Using stable isotopes to trace the sources and fates of nitrate within mixed land-use catchments on the Banks Peninsula New Zealand

A thesis submitted in partial fulfilment of the requirements for the degree of Master of Science in Environmental Science at the University of Canterbury

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Abstract

Increasing land-use change and land-use intensification over the last century has resulted in lotic systems being faced by an increasing intensity environmental. Potentially the most pervasive of these stressors is increased nitrogen runoff. However, understanding the affects of excess nitrogen (primarily nitrate) on lotic systems is severely complicated by the fact that multiple interacting stressors are associated with any given land-use and catchments will invariably contain multiple land-uses. Having analytical tools which can trace nitrogen as it is being cycled through the system is thus important for understanding the impacts of various land-uses on stream nitrogen-cycling. Stable isotopic analyses of $\delta^{15}N$ and $\delta^{18}O$ values within nitrate and $\delta^{15}N$ and $\delta^{13}C$ values within organic matter allow us to compare how nitrate in entering lotic systems and subsequently moving through them across multiple land-uses. In this study, I compared nitrate $\delta^{15}N$ and $\delta^{18}O$ and invertebrate $\delta^{15}N$ and $\delta^{13}C$ values within 35 streams across six land-uses (regenerating indigenous forest, horticulture, golf courses, dry stock agriculture, dairy agriculture and land invaded by exotic N-fixing legumes gorse (*Ulex europaeus*) and broom (*Cytisus scoparius*)) on the Banks Peninsula, New Zealand. Results showed that gorse and broom streams had significantly higher mean nitrate concentrations than all other land-uses (mean NO_3-N = 1.02 ppm, $P < 0.001$). Furthermore, nitrate $\delta^{15}N$ and $\delta^{18}O$ values demonstrated that this elevated nitrate was being fixed by the plants themselves as opposed to a land-use legacy effect. Overall, native regeneration sites had significantly lower nitrate $\delta^{15}N$ values than all other land-uses. Across all land-uses, except for regenerating indigenous forest, nitrate $\delta^{15}N$ and $\delta^{18}O$ values displayed positive covariation, indicative of biological fractionation. Results suggested that, at least within gorse and broom systems, this fractionation was primarily being driven by biological uptake as opposed to denitrification. However, the environmental parameters which had the greatest affects on these fractionation relationships differed

substantially between land-uses suggesting that the factors controlling nitrate removal were specific to the land-use environment.

Lotic invertebrate responses to land-use included a reduced dietary intake of coarse particulate organic matter (CPOM) across all sites relative to regenerating indigenous forest sites and a larger community trophic niche (range in $\delta^{13}C$ values) in dairy and gorse sites relative to native regeneration and dry-stock sites. Land-use change lead to invertebrates having less CPOM available and subsequently feed on a wider range on trophic channels. However, no clear relationships were observed with these invertebrate trophic responses and individual land-use stressors (i.e. nitrate concentration or light availability), suggesting that trophic responses resulted from complex interactions between these stressors much in the same way these factors interacted to affect nitrate removal. Nonetheless, average $\delta^{15}N$ values for the whole invertebrate communities were lower in regenerating indigenous forested sites than all other sites, indicating that land-use induced changes to in-stream nitrogen cycling leaves a $\delta^{15}N$ imprint on the invertebrate community.

These findings have identified a significant novel source of nitrate within the regional landscape while also providing a uniquely holistic insight into the ways in which land-use impacts nitrogen cycling and community responses within lotic systems. Although these finding have highlighted the complexity associated with relationships between land-use and lotic systems responses, they also demonstrate how multiple stable isotopic proxies can elucidate vital mechanistic information. With more widespread data collection in New Zealand, in the future stable isotopic studies will be able to be a significant management and research tool for tackling the challenges faced by environmental practitioners in the 21st century.

Chapter 1: Introduction

Land-use change is one of if the most pressing contemporary concerns facing freshwater managers (Sala et al. 2000; Allen 2004; Gruber and Galloway 2008). These changes are occurring both rapidly and frequently. Lotic systems are the aquatic interface with the terrestrial environments meaning that they are acutely susceptible to the impacts of land-use change (Carpenter 1992; Carpenter et al. 1998; Allen 2004; Dodds 2007). While there are numerous land-use stressors which can impact lotic systems, arguably the most pressing concern is nitrate loading (Carpenter et al. 1998). This is primarily because of two reasons: firstly, nitrogen is a growth limiting nutrient which can elicit drastic responses within various components of the ecosystem; secondly, technological advances in creating reactive nitrogen mean that anthropogenic supply is now potentially limitless (Galloway et al. 2003 Sala et al. 2000). Nitrogen is cycled through organic and inorganic (primarily nitrate within freshwater systems) states through ecosystems and can have significant ecological impact both in its organic form and its inorganic form. This means that it is difficult to identify where nitrate is coming from within a landscape and the various ways in which it is impacting the receiving lotic system. Furthermore, this is complicated by the fact that nitrogen-cycling interacts with other environmental variables which are affected by land-use change (Allen 2004; Gruber and Galloway 2008). Such a complex and pertinent issue requires analyses which are both comprehensive and detailed.

Stable isotopic analyses trace nitrogen ($\delta^{15}N$ values) as it is being cycled through a system (Robinson 2001; Finlay and Kendall 2007; Kendall et al. 2007). As a result, $\delta^{15}N$ values have been proposed as an integrated measure for quantifying land-use impacts of both organic and inorganic stages within the nitrogen-cycle (Robinson 2001). $\delta^{15}N$ values alone are unable to elucidate much information on

nitrogen-cycling; however when combined with multiple stable isotopic proxies (i.e. nitrate – $\delta^{15}N$ & $\delta^{18}O$ values as well as organic matter $\delta^{15}N$ & $\delta^{13}C$ values) the power to identify land-use impacts is substantially increased (Finlay and Kendall 2007; Kendall et al. 2007; Nestler et al. 2011). Dual isotopic investigations have previously been highly successful elucidating land-use effects on nitrate loading and ecosystem processing as well as trophic transfers on nitrogen within foodwebs. Although integrating stable isotopic analyses of nitrate – $\delta^{15}N$ & $\delta^{18}O$ values and organic matter $\delta^{15}N$ & $\delta^{13}C$ values have the potential to provide a holistic understanding of the sources and fates of nitrate within a mixed land-use catchment, such studies are notably scant in the literature. In this thesis I have used nitrate – $\delta^{15}N$ & $\delta^{18}O$ values and organic matter $\delta^{15}N$ & $\delta^{13}C$ values as well as measures of other environmental parameters to investigate how land-use is effecting the sources and fate of nitrate within-streams in a single ecoregion, the Banks Peninsula on the east coast of the South Island, New Zealand.

In this thesis I address this objective by firstly reviewing the current literature on the relationship between land-use and elevated nitrate levels in lotic systems in chapter 2. In particular I bring together the various threads of research, primarily those focusing on processes associated with cycling of nitrate and those focusing on transfers of nitrogen through the foodweb within-streams, and how stable isotopic analyses can address these problems. Chapter 3 then provides a description of the study area, the Banks Peninsula. In chapter 4 I then present water chemistry data from streams on the Banks Peninsula comparing six land-uses; native regenerating forest, land invaded by the exotic N-fixing shrubs gorse (*Ulex europaeus*) and broom (*Cytisus scoparius*), horticulture, golf courses, dry stock agriculture and dairy agriculture. Using nitrate – $\delta^{15}N$ & $\delta^{18}O$ values as well as a suite of other physicochemical parameters I explore the affects of land-use on nutrient concentration and nitrogen-cycling. Chapter 5 then uses $\delta^{15}N$ & $\delta^{13}C$ values of members of the lotic invertebrate community across the six land-uses to

explore how nutrient cycling changes manifest in the foodweb. An in depth focus on sites from native regenerating forest, gorse and broom, dry stock and dairy sites investigates specifically how invertebrate trophic interactions and their trophic niches respond to land-use. Finally in chapter 6 these investigations are broought together and some potential future research paths are suggested.

The two data chapters of this thesis are written as standalone manuscripts which are intended for submission to academic journals. This means that there are some inconsistencies in the use of terms between them (i.e. stream systems are primarily referred to as lotic systems in chapter 5) based on the intended audience. It also means that there is degree of repetition within the methodology and cited literature is referenced individually for each chapter. Additionally this meant that some data which warranted documentation fell outside the scope of the manuscripts; as a result there are three appendix sections which describe this omitted data. The first used isotopic niche indices to measure and compare the scatter of nitrate – $\delta^{15}N$ & $\delta^{18}O$ values between land-uses, the second shows longitudinal changes in nitrate concentrations and nitrate – $\delta^{15}N$ & $\delta^{18}O$ values down the length of five catchments, while the third describes a longitudinal enrichment relationship observed within invertebrates' $\delta^{13}C$ values. Other appendices show photographs of typical habitat present in different land-uses.

1.2 Literature cited

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Chapter 2: Bringing together the various thread of nitrogen cycling research: a review

2.1 Introduction

Land-use change is one of the primary drivers of the global decline in natural ecosystems and the services which they provide (Vitousek et al. 1997; Sala 2000; MEA 2005). Nowhere else is this more pronounced than in freshwater ecosystems (Carpenter 1992; Allen 2004; Woodward 2009). Land-use change impacts freshwater systems in a variety of ways such as reduced organic matter input, increased sedimentation, light and heat stress, toxins and increased nutrient runoff (Allen 2004). While all of these disturbances cause significant deleterious effects, increasing nutrient additions (specifically reactive nitrogen) is acutely important to freshwater systems. Freshwater systems are the primary vector for transporting pollutants into costal marine environments (Carpenter et al. 1992). Moreover, freshwater systems are biodiversity hotspots which provide integral ecosystem services to humans (Allen 2004; Woodward 2009). Impacts from nitrogen pollution include: the acidification of waterways and soil through the oxidation of ammonia (Carpenter et al. 1992; Gruber and Galloway 2008) nitrogen fertilization stimulating algal production, inducing eutrophication and leading to anoxic conditions as this algal biomass is metabolized by microbes (Dodds 2007); loss of species diversity through both competitive exclusion and degradation of environmental conditions (Sala 2000; Allen 2004); when directly induced in water in high concentrations, nitrate can cause methhaemoglobinemia which can be fatal be infant humans (Erisman et al. 2007; Gupta et al. 2008). Nitrate is the primary form of nitrogen pollution within freshwater systems (Galloway et al. 2004).

The ecological significance of nitrogen pollution is widely recognized. An ISI web search using "nitrogen pollution" returned over 9000 peer-reviewed articles and proceedings papers; of which ~70% were published in the between 2000 and 2010. The seminal works of Vitousek et al. (1997a & b) and

Galloway et al. (1994, 2004), with a combined citation record of 4384, have been instrumental in focusing the research into nitrogen pollution over the last decade and a half. These studies identified globally significant sources of reactive nitrogen and identified ecosystem impacts across terrestrial, freshwater and marine systems. However, this accumulation of knowledge has been hindered through the piecemeal way in which it has come together. The interdisciplinary nature of investigating the nitrogen cycle means that there have been many different approaches to describing associated phenomena; making it hard to compare finding from across disciplines. Bringing these strings of research together into an integrated approach represents a significant step forward in understanding the causes of nitrate impacts within freshwater systems (Galloway et al. 2003; Gruber and Galloway 2008).

Stable isotope analyses of the various components within a lotic nitrogen cycle hold significant potential for integrating these fields of research. Stable isotopic analysis traces the nitrogen containing molecules as they are transferred through the various components of the nitrogen cycle (Sharp 2007). Numerous studies have already used stable isotopic analyses within various areas of research (primarily identifying sources of nitrate and investigating trophic transfers within foodwebs) successfully (Finlay and Kendall 2007; Kendall et al. 2007); however, wider applications are still somewhat hindered by a lack of understanding of what is controlling natural variability within observed isotopic signatures (Finlay and Kendall 2007; Kendall et al. 2007; Martínez del Rio et al. 2009).

This review aims to highlight the current issues in understanding nitrogen cycling within freshwater systems and bring together the various strings of research related to nitrate pollution in freshwater systems. I will then demonstrate how stable isotope based studies present the most promising path forward. Current limitations to applying stable isotope based investigations will be discussed as well as

promising future directions. In this review I will focus primarily on aspects on nitrogen cycling at the catchment scale. Catchments represent discrete comparable ecosystems units and are also the scale at which environmental management is generally operated.

2.2 Sources of reactive nitrogen

The largest reservoir of global nitrogen is in the atmosphere; however this nitrogen is dinitrogen (N_2 gas) which is unavailable to life forms. This nitrogen must be hydrolisized or oxidized to become bioactive. At this stage it becomes available to flora and is cycled through ecosystems (fig. 1). Naturally this occurs during chemical reactions in lightning and biological nitrogen fixation (Galloway 2004). However today, globally the primary sources of bioactive nitrogen are synthetic fertilizer, fossil fuel combustion and biological nitrogen fixation (Galloway et al. 2004).

2.2.1 Synthetic fertilizer production

Synthetic fertilizer is produced through the Haber-Bosch process; in short, hydrogen and atmospheric di-nitrogen are exposed to an iron catalyst under high temperature and pressure conditions. For just over 100 years humans have been using Haber-Bosch nitrogen fertilizer as the primary source of nitrogen on agricultural land (Erisman et al. 2007; Erisman et al. 2008). Conservative estimates suggest that crop yield has increased by 30 – 50% over that time while nitrogen fertilizers are now feeding 44% of the world's population (Erisman et al. 2008). Currently humans use approximately 100 Tg N yr^{-1}, making it the largest source of bioactive nitrogen globally (Galloway et al. 2004). Ammonium-nitrate fertilizer is applied primarily to productive land meaning that direct deposition areas are solely associated with anthropogenic activities.

2.2.2 Fossil fuel nitrogen fixation

The combustion of fossilized organic matter in fuel releases bioactive nitrogen into the atmosphere that was otherwise locked up in the earth's crust; while, smaller quantities are also fixed from the atmosphere during the combustion process (Galloway et al. 2004). This bioactive nitrogen is emitted into the atmosphere after which it is then deposited on the earth's surface via rain and direct fallout. Although areas of high deposition are commonly associated with industrially intensive areas (i.e. Europe and North America), the interactions of wind and precipitation mean that deposition can occur on otherwise pristine areas (Vituosek et al. 1997). Currently, anthropogenic activities inadvertently produce approximately 21.5 Tg N yr^{-1} globally (Galloway et al. 2004).

2.2.3 Biological nitrogen fixation

Biological nitrogen fixation is the result of a select group of plants (primarily legumes) that have developed a symbiotic relationship with bacteria which allows them to fix their own nitrogen from the atmosphere (Vitousek et al. 2008; Galloway et al. 1995). Some algal species are also able to do this (Galloway et al. 2004; Ozinski et al. 2010). Although nitrogen fixed by plants will invariably be assimilated directly into the plant tissue, once that biomass has either been shed and decomposed or consumed through herbivory it becomes available to the rest of the ecosystem (Galloway et al. 2003; Magesan *in press*). Traditionally, biological fixation has been the primary source of bioactive nitrogen; Galloway et al. reports that 107 Tg N yr^{-1} is biologically fixed with 31.5 Tg N yr^{-1} of that being from cropping plants such as clover and soya.

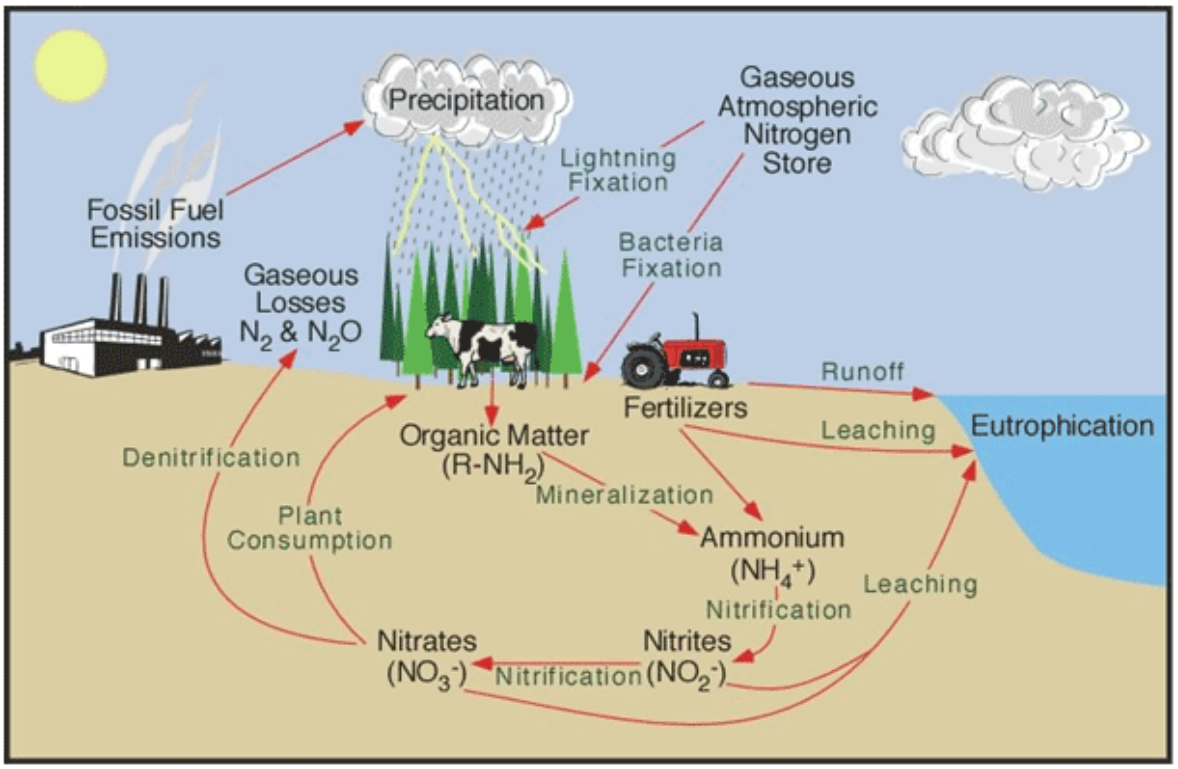

Figure 2.1: The Nitrogen Cycle. From: Pidwirny, M. (2006). Fundamentals of Physical Geography, 2nd Edition.

2.3 Understanding the impacts of increased nitrate loading on freshwater systems

All of these potential sources of reactive nitrogen have been linked with incidences of freshwater nitrogen pollution primarily in the form of nitrate (Carpenter et al. 1998). Nitrate pollution results in a variety of impacts within freshwater ecosystems (Galloway et al. 2003). In unperturbed systems with characteristically low nitrate concentrations, nitrate that does enter the water is rapidly taken up by photoautotrophs and heterotrophic bacteria; then becoming organic nitrogen (i.e. amino acids, DNA and protein) available to higher order consumers. Subsequently, the majority of bioactive nitrogen will be locked in organic matter. However, deviations from this state will invoke accumulation on nitrate within

the waterway (Allen 2004). Measuring nitrogen stress within freshwater systems is best achieved through analyzing nitrate as opposed to other forms of bioavailable nitrogen.

Howarth et al. (1996) demonstrated a strong correlation between nitrate export and the percentage of anthropogenic land-use within the major catchments discharging into the North Atlantic Ocean. This excess nitrate was primarily entering the waterways via diffuse sources (i.e. surface run-off and soil transport) as opposed to point sources (i.e. discharge pipes). More recently similar relationships between anthropogenic land-use and elevated nitrate concentrations have been reported from lotic systems around the world, with diffuse sources being the primary contributors (Carpenter et al. 1998; Ventrua et al. 2008). New Zealand is no exception with diffuse sources also being the primary contributor of nitrate lotic systems (Davies-Colley and Wilcock 2004; Larned et al. 2004; Parfitt et al. 2008). Diffuse sources of pollution are problematic to quantify as they enter the water system over a large spatial area.

Although estimations (Galloway et al. 2004) and observations (Howarth et al. 1996) have been successfully done for diffuse sources at the global and inter-continental scale, the finer scale of accuracy required for application at the watershed and catchment scale have proven more difficult. Aspects confounding this are: landscape heterogeneity; longitudinal spatial-autocorrelations of land-uses within catchments masking potential influence of position within a catchment (Dodds and Oaks 2007; Peterson et al. 2001); the effect of seasonality on both the relative importance of nitrate sources and the aquatic ecosystem response to it (Johannsen et al. 2008; Piatek et al. 2009); the probable occurrence of multiple sources of nitrate associated within a single land-use (Galloway et al. 2003; Kellman and Hillaire-Marcel 2003; Kendall et al. 2007; Robinson 2001).

Understanding, and identifying, the origins of nitrate within a multi-land-use landscape has been an integral issue both, theoretically and practically, for over two decades across the spectrum of environmental sciences globally (Bernot and Dodds 2005; Galloway et al. 2003; Kellman and Hillaire-Marcel 2003; Kendall et al. 2007). While correlative relationships drawn between land-use and nitrate concentrations have been greatly beneficial for environmental management, confounding factors may be masking the actual causal relationship in some cases (Legendre 1993; Allen 2004). Headwater reaches are shown to respond differently to nitrate additions than those further down (Peterson et al. 2001: Dodds and Oaks 2007). Anthropogenic land-uses are situated within the lower reaches most catchments making the two relationships indistinguishable. Recently previously unrecognized significant nitrate sources (invasive N-fixing legumes) have been identified (Goldstein et al. 2009; Mineau et al. 2011; Magesan *In press*). This calls into question some previous held assumptions of the primary sources of nitrate within some land-uses.

These findings highlight that N-cycling within catchments is a highly complex biological system, making attempts to partition potential nitrate sources a considerable challenge. This complexity justifies the variety of approaches that have been employed throughout the literature to understand the origins of nitrate and the mechanisms controlling its presence. A more mechanistic understanding of what controls the distribution of nitrate within the environment can overcome this challenge (Bernot and Dodds 2005; Carpenter et al. 1998). Mechanistic information is then able to be fed into robust models predicting relative source contributions (Mulholland et al. 2008; Tiemeyer et al. 2007). Using naturally occurring markers to trace sources is another, more direct, way to distinguish nitrate origins. These include fecal coli forms (Meays et al. 2004), pharmaceuticals (Zhao et al. 2011) and natural abundance of stable Isotopes (Xue et al. 2009). While all of these fields present exciting prospects for aiding our

understanding of the contributions of nitrate pollution, this review will focus on the significance and potential of stable isotopic source tracking analyses. Stable isotopic analyses are unique in that they trace the actual body of nitrogen (opposed to an associated proxy) as it is moving through the cycle. This makes it possible for both the origins of the nitrate and mechanisms of delivery and transformation can be recognized.

2.4 Using stable isotopic to investigate nitrogen cycling

Stable isotopic analysis measures the ratios of a common light isotope and a rare heavy isotope of an element found within a chosen material (Sharp 2007). These ratios are then expressed relative to the ratio of an international standard reference material. For example, the isotopic signature of nitrogen ($\delta^{15}N$) is shown as:

$$\delta^{15}N(‰) = \left(\frac{{}^{15}N/_{14}N\ (Sample)}{{}^{15}N/_{14}N\ (Standard)} - 1 \right) \times 1000$$

where a +1‰ change represents a one part per thousand enrichment in ^{15}N within the sample. The reference materials used for these elements are air for nitrogen, Vienna Standard Mean Ocean Water (VSMOW) for oxygen and Vienna Pee Dee Belemnite (VPDB).

The three primary elements commonly of interest to nitrogen cycling studies are nitrogen ($\delta^{15}N$), Oxygen ($\delta^{18}O$) and Carbon ($\delta^{13}C$). $\delta^{15}N$ & $\delta^{18}O$ values of nitrate are used as indicators of processes occurring within the nitrogen cycle and the conditions under which the nitrate was formed (i.e. origins) (fig. 2). $\delta^{15}N$ & $\delta^{13}C$ on the other hand are used to elucidate trophic transfers; the way in which nitrogen is cycled through ecological communities. Nitrate – $\delta^{15}N$ & $\delta^{18}O$ values and $\delta^{15}N$ & $\delta^{13}C$ values of organic

matter are primarily controlled by kinetic fractionation during metabolic processes. Kinetic fractionations are process driven, permanent alterations in the relative ratios of heavy and light isotopes within the elemental pool. Before these assumptions can be applied to real-world studies, the isotopic signatures of the potential sources (as well as associated variation) and the fractionation rates of relevant processes within the nitrogen cycle must be understood.

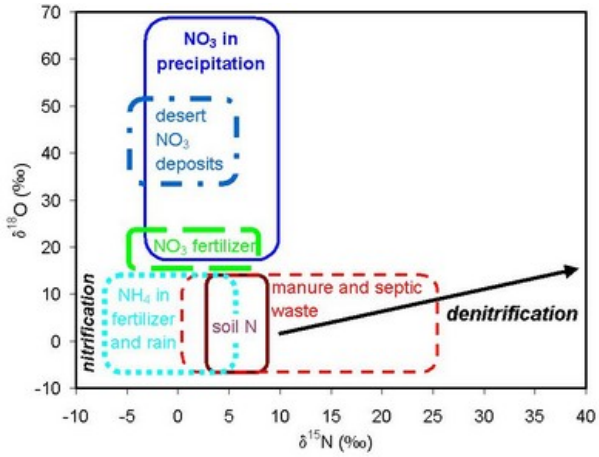

Figure 2.2: characteristic patternns in nitrate source δ15N & δ18O isotope signatures. From Kendall et al. 2007

Xue et al. (2009) synthesized the last decade of $\delta^{15}N$ $\delta^{18}O$ isotope source tracing studies. Figure 3 below demonstrates the range of values observed for both $\delta^{15}N$ $\delta^{18}O$ for a range of nitrate sources taken from studies conducted around the world over the last ten years.

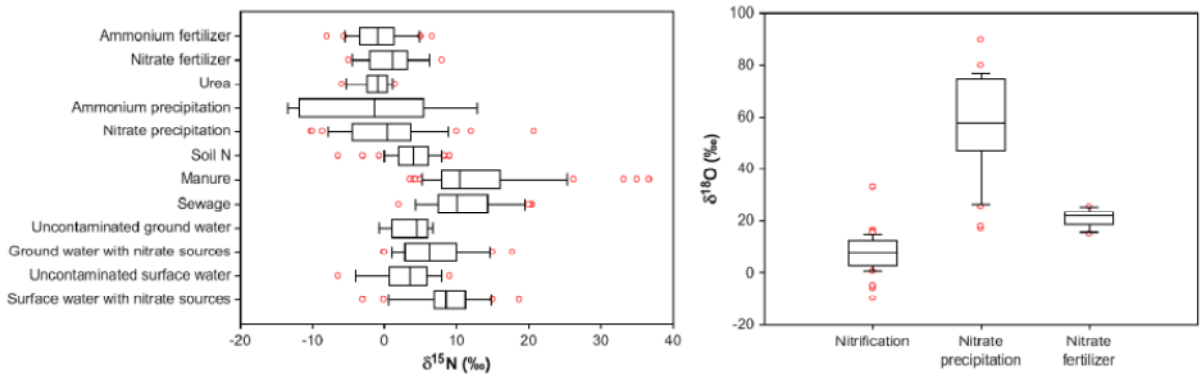

Figure 2.3: ranges in nitrate source δ15N (left) & δ18O (right) isotope signatures taken from numerous field studies. From Xue et al. 2009

$\delta^{15}N$ is a commonly used proxy for identifying the trophic level; the common rule of thumb being there is a 3‰ enrichment in $\delta^{15}N$ for each trophic transfer (Martínez del Rio et al. 2009). Therefore $\delta^{15}N$ can enable us to estimate the trophic level of sources of nitrate contamination (i.e. grazing stock vs. human derived effluent) (Xue et al. 2009).When investigating potential nitrate sources, we would expect to see nitrate that was sourced from atmospheric deposition, biological N-fixation and synthetic fertilizer to have $\delta^{15}N$ signatures of approximately 0‰ (with a reported range, however, of up to 20‰) as they are all sourced directly from the atmosphere (Xue et al. 2010). Whereas manure and sewage sources have been subject to metabolic fractionation resulting in their signatures being > 0‰. Values for sewage and manure have been reported to average 8‰ with a range of up to 40‰ (Xue et al. 2009). The signature of effluent (combined manure and urea) con be complicated because urea is isotopically deplete ($\delta^{15}N$ = 0± 5‰) relative to manure (Xue et al. 2009). This is due to metabolic fractionation favoring N^{15} to remain in the lower energy state of organic matter while N^{14} is more likely to be liberated, through metabolism, into urea (Martínez del Rio et al. 2009). Kellman and Hillaire-Marcel

(2003) evaluated the use of $\delta^{15}N$ signatures as indicators of agricultural effluent derived nitrate and concluded that $\delta^{15}N$ values alone were insufficient to identify nitrate contamination within mixed agricultural watersheds. This was largely due to in-stream and soil processes further fractionating the nitrate body and masking the source signatures. It is evident from this that other tracers are necessary to elucidate sources.

While NO_3 – $\delta^{15}N$ is a good indicator of trophic position, NO_3 –$\delta^{18}O$ will indicate processes and origins behind the formation of the nitrate. The three major sources of nitrate: nitrification, atmospheric formation (lightning and the consequence of fossil fuel combustion) and the Haber-Bosch process all create a distinct $\delta^{18}O$ signature on the nitrate (fig. 2.2). A dual NO_3 – $\delta^{15}N$ & $\delta^{18}O$ analysis has the potential to account of the source variation described by Kellman and Hillaire-Marcel (2003).

Described NO_3 –$\delta^{18}O$ source signatures are based on averages taken from a variety of studies across the world. Due to inter-system differences, the variation in source isotopic signatures is large. For example, Nanus et al. (2008) reported isotopic values of precipitation nitrate within the Rocky Mountains (West Coast USA) of $\delta^{15}N$ = -6.6 – 4.6‰ compared to -10 - 10‰ for the global average NO_3 – $\delta^{15}N$ (precipitation) and $\delta^{18}O$ = 71 – 78‰ compared to 20 – 78‰ for the global range. Anisfeld et al. (2007) reported a similar range of $\delta^{18}O$ values and $\delta^{15}N$ = -4 – 1‰ for precipitation derived nitrate from two catchments within Connecticut (East Coast USA). They also reported values of human effluent ranging from $\delta^{15}N$ = 4 – 18‰ and $\delta^{18}O$ = -3 – 4‰ compared to the global averages of $\delta^{15}N$ = 1 – 20‰ and $\delta^{18}O$ = -5 – 18‰. Similarly, other research reporting $\delta^{15}N$ $\delta^{18}O$ at a local and regional scale report smaller ranges in their values. A meta-analysis of previous studies would likely indicate a positive relationship between the scale of the study and the range of isotopic values reported; however inconsistencies with data reporting prevents this analysis being conducted here.

While we are able to conclude from this that various nitrate sources do exhibit identifiable $\delta^{15}N$ & $\delta^{18}O$ isotopic signatures, many land-uses will be receiving this nitrate through a variety of pathways and potentially from multiple sources. As a result, a sound understanding of the mechanisms underlying these isotopic signatures is central to providing nitrate source apportionment for a given freshwater system.

2.5 Mechanisms causing nitrate isotopic fractionation

As mentioned, a variety of kinetic fractionation effects occurring in the various pools within the N-cycle (Kendall et al. 2007). These kinetic fractionations are important in enabling us to partition N into various pools and source pathways, yet the simultaneous occurrence of multiple fraction effects can make understanding the mechanisms and partitioning sources challenging.

When nitrate pollution is from a diffuse source, it means that there are multiple potential channels through which it may have come to be present in that waterway. For example, animals grazing on a riparian margin may be the primary source of the stream's nitrate. This nitrate may be the result of the animals urinating directly into the waterway, in which case the primary nitrogen input would be urea which was then nitrified within the water column. Alternatively, feces deposited directly into the water would supply, primarily, organic nitrogen which would then be hydrolysed into ammonia and subsequently nitrified within the water column. If urine and feces were deposited on the riparian margin they would then enter the stream via either surface run-off or leaching through the soil. If however, they leached through the soil, the biogeochemical transformations would occur within the soil resulting in nitrate entering the stream; whereas surface runoff would likely contain significant portions of nitrate, it is likely that a large portion of the input would be ammonia. Each of these potential nitrate

pathways has been theorized, and proven in some instances, to leave an isotopic imprint on either the $\delta^{15}N$ or $\delta^{18}O$ signatures of the in-stream nitrate (Kendall et al. 2007; Xue et al. 2009).

2.5.1 Nitrification

Nitrification is a complex process involving multiple environmental variables. The isotopic controls on nitrification have been discussed in depth by Kendal et al. (2007); hence, here I will briefly summarize this information and present some recent developments. Nitrification is a metabolic reaction which is generally considered to follow the equation:

$$NH_3^+ + CO_2 + 1.5\ O_2 + \text{Nitrosomonas} \rightarrow NO_2^- + H_2O + H^+ \rightarrow NO_2^- + CO_2 + 0.5\ O_2 + \text{Nitrobacter} \rightarrow NO_3^-$$

In which, on average, water molecules will contribute two oxygen atoms to the nitrate product and one will come from the air. This means that isotopic variation within nitrification are largely due to isotopic variation in water and air ($\delta^{18}O$) and ammonium ($\delta^{15}N$).

2.5.2 Causes of variation in $\delta^{18}O$ values

Because water and air have characteristic $\delta^{18}O$ isotopic signatures, nitrate – $\delta^{18}O$ values can provide insight into the environment where nitrification has occurred. Nitrification within the soil environment has been shown to have a distinct higher nitrate – $\delta^{18}O$ signature than nitrification occurring within the stream channel. This is because evaporstanpiration within the soil discriminates against ^{18}O leaving the soil water with relatively high $\delta^{18}O$ signature; a signature which is then imprinted on the nitrate produced within the soil. So far however, this mechanism has only been demonstrated in arid environments (Böhlke et al. 1997). Several caveats to nitrification isotopic signature relationships have, however, been highlighted. Seasonal variation in the source of the rainfall can impact the observed NO_3-

$\delta^{18}O$ if the H_2O- $\delta^{18}O$ is unknown (Wassenaar 1995). Moreover, the assumption that air O2- $\delta^{18}O$ = +23.5‰ (Kroopnick and Craig 1972) may not hold true for soil environments as soils may be enriched in $\delta^{18}O$ as the result of respiration (Kendall 1998). While these uncertainties can be accounted for with relatively simple measurement (recording the isotopic composition of the air and water within the environments), deviations from the assumed 33.3% contribution of water to the nitrate molecule's oxygen can impact isotopic interpretation (Kool et al. 2007). Kool et al. (2007) showed that a broad range of H_2O- $\delta^{18}O$ contribution can exist (12 – 100% H_2O assimilation) between different nitrifying bacterial cultures with no clear understanding of the causal mechanisims for this variation. Casciotti et al. (2010) demonstrated inter-species variation accounted for a 10‰ range in nitrate – $\delta^{18}O$ values from nitrification in natural environments. Nestler et al. (2011) provide evidence to suggest that nitrification occurring within incubated soils was in the range of NO_3-$\delta^{18}O$ ≈ 80% H_2O derived O while the product of lake nitrification producing ~ 83% H_2O derived O of NO_3-$\delta^{18}O$.

2.5.3 Causes of variation in $\delta^{15}N$ values

Reported ammonia $\delta^{15}N$ values also range widely (Robinson 2001). Understanding this variation is important as nitrification of aerial ammonia deposition can be a significant environmental source of nitrate. Robinson (2001) used an example of a Sub Antarctic penguin breeding colony to show that ammonia volatilization can lead to enrichment of the guano N-pool within the colony (>10‰) while the $\delta^{15}N$ deplete ammonia will rain out on the upper slopes of the island. Understanding the role of ammonia volatilization within an environment can significantly reduce the noise associated with ammonia sourced nitrate. Within my study area there are no current avian breeding sites. , urea excreted by stock animals will be rapidly converted to ammonia. Thus, the duration of time the urea is exposed to the atmosphere prior to being taken-up by plants or nitrified will have an influence on the

$\delta^{15}N$ signature of the final nitrate produce with longer atmospheric exposure leading to a more enriched N-pool. Equally, urea deposition directly into the water column will reduce the fractionation caused by volatilization.

2.5.4 Denitrification

As mentioned earlier, denitrification is a kinetic fractionation process that will preferentially remove the lighter N & O atoms from the nitrate pool leaving the remaining nitrate enriched in both $\delta^{15}N$ & $\delta^{18}O$. This results in denitrification imprinting a positive linear enrichment line, with a gradient commonly between 0.5 and 1, when nitrate data are plotted out in $\delta^{15}N$ – $\delta^{18}O$ isotopic space. Hence, deviations from the linear relationship can be seen as evidence of additional sources. The mechanisms controlling the observed slope of the denitrification line are still not entirely understood; while laboratory experiments demonstrate that mono cultures of denitrifying bacteria will result in a 1:1 enrichment slope, this is commonly not observed in the, now large, collection of field studies (Kendall et al. 2007). While it is intuitive that natural denitrification will be the result of a multi-species microbial community, quantifying this presents large technical challenges (Kool et al. 2007). It was been suggested that denitrification using the 'auxiliary denitrification' enzyme (present in many bacteria species) may in fact produce a slope of 1:2, meaning that variation in the slope is the result of proportional input from two denitrification end-members (Kendall et al. 2007). Another possibility in the variation in the observed slope may be due to the difference of benthic vs. water column denitrification. Differences have been observed between denitrification slopes in pelagic and benthic denitrification environments within marine ecosystems (Sigman et al. 2003); however, Kendall et al. (2007) postulated that the observed lower slope in benthic environments is likely to be due to mixing of the denitrified sediment pore water with non-denitrified overlying pelagic water. Nonetheless, denitrification can mask nitrate

source signatures. When denitrification is heterogeneous within the environment, it can result in two sources that were originally isotopically distinct (i.e. legume fixed-N and stock effluent) having overlapping $\delta^{15}N$ and/or $\delta^{18}O$ signatures. Anisfeld et al. were able to explain 88% of the variation in $\delta^{15}N$ $\delta^{18}O$ values for nitrate derived from human effluent by accounting for the level of denitrifiction the effluent was subjected to during sewage plant processing; $\delta^{15}N$ was enriched twice as much as $\delta^{18}O$.

2.5.5 Nitrate uptake

Detecting denitrification relationships is confounded by in-stream biological nitrate uptake which has also been shown to demonstrate a ≈ 1:1 fractionation relationship (Granger et al. 2004; Deutsch et al. 2009; Granger et al. 2010). Further, chemoheterotrophic uptake (i.e. bacteria and fungi) produces a 2:1 relationship (Granger et al. 2010). Deutsch et al. (2009) paired stable isotopic relationships with chlorophyll a concentrations to distinguish N-uptake from denitrification. In the absence of chlorophyll a data, the primarily method for discerning the two processes has been to compare the relationship of $\delta^{15}N$ or $\delta^{18}O$ values against the nitrate concentration. A positive relationship indicates that assimilation is the primary nitrate sink while a negative relationship indicates that denitrification is the primary sink (Granger et al. 2004; Johansen et al. 2008; Kendall et al. 2007). The positive $\delta^{15}N$ – nitrate concentration relationship with uptake is due to a higher degree of discrimination (i.e. preferential ^{14}N uptake) when nitrate is more readily available. Granger et al. (2010) stress the importance of the difference in the kinetic fractionation relationships between autotrophs and heterotrophs and highlight the need for further investigation into this area.

The controls of kinetic fractionation within the formation and processing of nitrate are numerous and complex; various distinct processes can result in indistinguishable isotopic signatures. However causative relationships can be made when other parameters are included in analyses or processes can

be spatially or temporally isolated. In order to elucidate source contributions within catchments' nitrogen budgets these multiple parameter analyses are required.

2.6 Applying nitrate stable isotopic data to source apportionment

An over reliance on stable isotope data may lead to erroneous conclusions. However, extensive reviews by Nestler et al. (2011) and Xue et al. (2009) demonstrate that, when used in conjunction with other chemical and hydrological data, nitrate $\delta^{15}N$ & $\delta^{18}O$ data can provide important information on the sources and mechanisms of surface water nitrate pollution.

Other measures such as soil properties and in-stream parameters can help us further elucidate these mechanisms. All studies identified which were able to make conclusive statements about the contribution of sources to a system's nitrate budget coupled nitrate isotopic data with other parameters (Anisfeld et al. 2007; Chang et al. 2002; Goodale et al. 2009; Mitchell et al. 2006; Nanus et al. 2008; Ohte et al. 2010; Piatek et al. 2009; Seiler 2005). On the other hand, studies which relied more heavily on nitrate stable isotopic data were only able to draw conclusions on mechanisms controlling the nitrate budget within the study systems (i.e. origin of nitrification and the degree of denitirification and assimilation) (Ganger et al. 2008; Itoh et al. 2011; Johannsen et al. 2008; Sigman et al. 2003; Schwarz et al. 2011). Of the eight aforementioned studies which were able to discern between sources, were investigating systems with two nitrate source end-members of which one was atmospheric nitrate (characterized by a distinctively high (>30‰) nitrate - $\delta^{18}O$ signature A variety of hydrological and chemical parameters have been employed to elucidate Land-use relationships including H_2O – δ^2D & $\delta^{18}O$ and N_2O – $\delta^{15}N$ & $\delta^{18}O$ (Ohte et al. 2010), $\delta^{11}B$ used as a conservative tracer (Seiler 2002) and SO_4^{2-} – $\delta^{34}S$ or $\delta^{18}O$, pH as well as trace element such as Al Si and Cl (Mitchell et al. 2006). One study investigating more than one other non-atmospheric source of nitrate which was able to show source

apportionment using nitrate stable isotopes (Chang et al. 2002) reported Land-uses having "overlapping but moderately distinct isotopic signatures".

Agricultural surface water systems have been specifically identified as lacking studies which can conclusively demonstrate source partitioning within the nitrate pool (Nestler et al. 2011). The major potential nitrate sources have been generically identified to be the mineralization of soil organic matter, crop N-fixation, atmospheric deposition, fertilizer, and stock effluent. Recently Zhao et al (2011) used stable isotopic data paired with measurements of pharmaceuticals and pesticides to partition urban fertilizer runoff and municipal waste contamination source contributions. While such analyses are currently expensive and not common place, it does provide a positive direction for future studies.

While being a powerful analytical tool nitrate $\delta^{15}N$ & $\delta^{18}O$ data are not an environmental 'silver bullet' and are best accompanied with supporting physicochemical and hydrologic data (Xue et al. 2009). Nitrate stable isotopic data does provide strong insight into nitrogen cycle processes; while analysing other environmental variables allows the location of these nitrogen cycle processes within the system (i.e. riparian habitat vs. stream channel) to be isolated. Identifying where certain processes are occurring within the system provides strong evidence for the relative contribution of various nitrate sources (Robinson 2001). Such parameters include isotopic data for the soil which can help constrain the soil organic matter mineralization end-member (Goodale et al. 2009; Schwartz et al. 2011); H_2O – δ^2D & $\delta^{18}O$ and physicochemical data can further partition in-stream and soil nitrification (Itoh et al. 2011; Johannsen et al. 2008; Ohte et al. 2010); information on stocking densities and fertilizer application rates (specifically varying at rates independently of each other) allows the recorded stream nitrate isotopic values to be validated against a source input gradient.

A clear mechanistic understanding of the nitrogen cycle processes involved in creating and transporting nitrate to a lotic water body is essential to achieving isotopic partitioning of nitrate sources within freshwater systems. To obtain this, a sound theoretical understanding of how the various components within the nitrogen cycle interact is required. So far this review has just covered isotopic characteristics associated with inorganic phases of the nitrogen cycle; however significant quantities of nitrogen are transferred through the floral and faunal communities within the nitrogen cycle. Organic nitrogen pool and transfers within the nitrogen cycle are often neglected from research (Galloway et al. 2003). However, to truly understand the causes of, and how ecosystems respond to, land-use induced nitrogen pollution all aspects of the nitrogen cycle must be accounted for.

2.7 The role trophic interactions within the nitrogen cycle

During recent years, there have been a number of studies that have shown structural and functional responses of stream invertebrate and fish communities to various perturbations associated with anthropogenic land-use disturbances (Allen et al. 2004 and references therein). Invertebrate responses are specifically well documented as they are commonly used as ecosystem health indicators both in New Zealand and internationally (Meyer 1997, Winterbourn 2004). Nutrient concentrations, specifically nitrate, have been the primary focus of many of these studies. There is now conclusive evidence to show that nitrate pollution can cause loss in diversity of stream communities (Frost et al. 2002; Hall et al. 2001; Singer and Battin 2007); change feeding interactions between consumers and resources within the community (Davis et al. 2010; Grover 2002; Frost et al. 2002); alter the balance of respiration and primary production (Dodds 2007; Ventura et al. 2008); impact important ecosystem functions (Antón et al. 2011; Benstead et al. 2009; Bernot and Dodds 2005; Grover 2004; Ventura et al. 2008).

Conversely to the aforementioned studies, there are a growing number of studies demonstrating that communities can alter the nutrient concentrations of their environments. The movement of animals can redistribute nutrients within the environment (often against the common, passive gravitational flows) creating productivity hotspots changing local nutrient dynamics (Ballenger and Lake 2006; Loreau and Holt 2004; Merleau et al. 2010; McIntyre et al. 2008). This research has largely stemmed from the development of the ecosystem subsidies hypothesis which describes how ecosystems are linked through the transfer of nutrients and other resources (Polis et al. 1997). Further to this, it was also been shown that interactions associated with other Land-use change disturbances can impact the ecosystems' response to the subsidy (England and Rosemond 2004; Tiegs et al. 2008). As an example, Tiegs et al. (2008) demonstrated that increased sedimentation induced by forest harvest changed the response of biofilm to spawning salmon in Alaskan streams. The increased sedimentation resulted in spawning salmon becoming bioturbators; mobilizing sediment, decreasing light penetration, subsequently decreasing biofilm nutrient uptake and productivity. Conversely in the absence of forestry induced sedimentation, the presence of spawning salmon provided biofilm with a nutrient subsidy increasing productivity.

Floral and faunal communities can be integral components of regional nitrogen cycles through a variety of ways; thus, which incorporate foodwebs (the primary matrix of ecological communities) into nitrogen cycling studies presents a fruitful area of research. The benefits of this integrated approach are beginning to be more widely recognized (Galloway et al. 2003; Marcarelli 2011). Galloway et al. 2003 devised the concept of the nitrogen cascade, which is used to describe how a single atom of reactive nitrogen can have multiple environmental consequences as it moves through a system. It was also suggested that the path in which this hypothetical nitrogen atom took through the system will greatly

alter its affects. Further to this, the form in which nutrients enter the stream foodweb can have drastic impacts on ecosystem response. An example is by changing the primary input of nitrogen into a stream from organic matter (i.e. leaf litter) to nitrogen runoff (inorganic) change the stream invertebrate community from being based on allochthonous production and feeding on autochthonous production. This may then result changes in the invertebrate community composition, having flow on effects through the foodweb (Marcarelli et al. 2011). Being able to trace nitrogen through these trophic exchanges will proved an importance causal link between traditional nutrient cycling responses and ecological community responses to land-use disturbances.

Stable isotope analyses represent powerful way to amalgamate the understanding of organic and inorganic components of the nitrogen cycle in lotic ecosystems. For over two decades $\delta^{15}N$ $\delta^{13}C$ stable isotopic analyses have been used within ecology to elucidate trophic interactions. The two primary assumptions to stable isotopic foodweb ecology are i) that trophic transfers (i.e. one organism consuming another) results in a ~3.4 ‰ enrichment in the consumer's $\delta^{15}N$ signature and a ~1 ‰ enrichment in the consumer's $\delta^{13}C$ signature (Post 2008) and ii) that differing photosynthetic pathways result in characteristically different $\delta^{13}C$ signatures (Marshal et al. 2007). Resultantly, when plotted out in isotopic space, $\delta^{13}C$ signatures will indicate trophic source while $\delta^{15}N$ signatures are a reference for trophic level.

Using these characteristic fractionation assumptions has lead to an extensive amount of research investigating nutrient flows through foodwebs. Amongst the best examples of these are studies which have taken advantage of ^{15}N enriched marine nutrients to trace marine derived nutrient subsidies through lotic communities (Anderson and Polis 1998; Harding et al. 2004; Lessard and Merritt 2006). However tracing nutrient from less isotopically distinct sources through foodwebs (i.e. various terrestrial

sources) can be more problematic due to high isotopic signature variability. Understanding this variability has subsequently motivated an increase in related research (Martínez del Rio et al. 2009). When accounted for, findings from these studies will reduce background variation within isotope signatures of ecosystem components and allow for more sources to be distinguished and traced.

2.8 Sources of variation in invertebrate stable isotopic signatures

A lotic invertebrate community generally consists of an array of organisms feeding with differing metabolic systems. Multiple organisms feeding on the same resource are likely to have different $\delta^{15}N$ & $\delta^{13}C$ signatures. This is largely due to i) variation in the rate at which organisms incorporate their diet's isotopic signature over time ii) their inherent isotopic discrimination factor ($\delta^{15}N_{(body)} - \delta^{15}N_{(Diet)}$ expressed as Δ) . The factors influencing isotopic incorporation rates are poorly understood; however Martínez del Rio et al. (2009) suggest body size, growth and protein turnover are important. The causes of variation in discrimination factors are similarly not entirely understood. Results from modeling indicate that $\Delta^{15}N$, will decrease with increasing the quality and quantity of protein in the consumers' diet, and also increase during times of fasting (Martínez del Rio et al. 2009). Alternatively, a meta-analysis identified that: $\Delta^{15}N$ decreased significantly with decreasing trophic resource C:N ratios (i.e. relatively more nitrogen); detritivores yielded significantly lower $\Delta^{15}N$ than grazers; organisms with ureotelic and uricotelic forms of waste excretion waste had higher $\Delta^{15}N$ than those which were ammonetelic (Vanderklift and Ponsard 2003). The commonalities between the two sets of findings are encouraging (i.e. increasing the diet's C:N in analogous to increasing the protein content), however both studies point out that there are uncertainties that need further addressing.

Lotic communities are able to be classed in a variety of different ways. These include separating the community into taxonomic classes, functional feeding groups or broad land-use habitat classifications.

These categories occur over differing levels of organization meaning that a meta-analysis comparing trophic discrimination factors between the levels has not been achieved to date (Martínez del Rio et al. 2009). Nonetheless, useful comparisons can still be made from the literature. Zambrano et al. (2010) specifically addressed intra-system variability within highly modified systems; showing that high levels of intra-community stable isotopic variation can occur within highly polluted systems. However, community stable isotopic signatures were still significantly different to communities from reference sites. Vanderklift and Ponsard (2003) found that crustaceans' $\Delta^{15}N$ were generally lower than those of other taxonomic classes of animals. Functional feeding group differences were also shown in that detritivores had low $\Delta^{15}N$ Relative to the other analysed functional groups (Predators, grazers, and filter feeders)

Systematic fractionation effects can significantly vary within the levels of organization commonly used to study lotic communities. While some of this variation is understood and can be accounted for (i.e. food source nutritional quality and taxonomic classification) other factors are currently unaccounted for, representing statistical noise. Validating assumed relationships between invertebrate and resource stable isotope signatures prior comparing communities between land-uses classes will increase potential predictive power and help avoid potentially erroneous conclusions. Combining stable isotopic values of lotic invertebrates with those of other ecosystem components will provide an opportunity to connect reactive nitrogen sources with plant uptake and subsequent movement (and recycling) through the foodweb.

While Galloway et al. (2003) discuss nitrogen flow pathways in a broad sense, emphasizing inorganic reactive phases; little emphasis has been placed on nitrogen cascades at the level of a community and trophic interactions (Marcerelli et al. 2011). Although it hasn't been specifically addressed, examples do

exist within the literature demonstrating that the form in which nitrogen is entering the stream impacts the community responses (Baxter et al. 2005; Leroux and Loreau 2008; Marczak et al. 2007; Polis and Holt 1996; Poils et al. 1997). Davis et al. (2008) Demonstrated that long term nutrient additions to a headwater stream increased the competitive advantage of large bodied grazers as they were able to better utilize the elevated levels of in-stream primary productivity. However the large bodied consumer was unable to be predated upon by the secondary consumers within the system. This resulted in the movement of nitrogen within the foodweb being truncated due to a source change (organic matter inputs vs. nitrate fertilizer) which is likely to impact the storage and redistribution of reactive nitrogen within the system (Loreau and Holt 2004). Measuring the relative makeup of a consumers' diet allows for the construction of foodweb nutrient flow paths (Marcerelli et al. 2011).

2.9 Using stable isotope mixing models to understand trophic interactions

Isotope mixing models present a powerful tool for assessing the relative contribution of multiple trophic sources to a consumers' diet (Phillips and Gregg 2003). Traditionally mixing models have been confounded by only being able to measure *n*+1 potential sources where *n* is the number of variables put into the model. For example, when using $\delta^{15}N$ $\delta^{13}C$ as model inputs, you could only look at contributions of three potential sources to a consumer's diet. However, the number of potential trophic resources in many stream systems exceeds three. Recent advances in mixing models (IsoSource and SIAR) allow for source apportionment with >*n*+3 sources by giving a probabilistic range of potential source contributions. Uncertainty with potential sources can be reduced through incorporating non-isotopic variables into the model (Phillips et al. 2005; Parnell et al. 2010) such as resource nutrient content and spatiotemporal grouping factors (sources that co-occur in space and time are more likely to form a mixture than those which are spatiotemporally disparate). Using stable isotope mixing models to

understand foodweb transfers will elucidate the flow of nitrogen through the organic phases of a stream's nitrogen cycle.

One of the major advantages to applying the described stable isotope techniques is their general applicability across multiple freshwater systems, and ecosystems generally for that matter (Post et al. 2002; Martínez del Rio et al. 2009). Hence, it is logical to be comparing units which are also applicable across multiple freshwater ecosystems when investigating land-use effects. As mentioned, when investigating trophic interactions in-stream communities, numerous studies have categorized animals into broad groups by their primary feeding mechanism (i.e. functional feeding groups). This has proven useful in elucidating land-use induced changes in community composition and trophic interactions within lotic communities in previous studies (Sandin and Solimini 2009). By definition, the various functional feeding groups within lotic communities have stronger associations with certain resources than others (i.e. grazers commonly have rasping mouthparts for feeding on benthic algae and films whereas filter feeders filamentous appendages or build webbed structures for catching fine particulate organic matter. However, Winterbourn (2004) warns against an overreliance on assumed feeding groups for certain taxa as these may vary at life stages and available resources. Nonetheless, once validated, functional feeding grouping presents a powerful way to investigate nitrogen flows through stream communities.

While mixing models allow for observing changes in the movement of nitrogen through a foodweb, recent developments in other tools used within biogeochemical-ecology allow us to compare broad, community-wide foodweb changes associated with Land-use changes. Stable isotopic niche indices allow us to quantify and compare changes in the breadth and vertical structure of whole communities in a simplistic manner (Layman et al. 2007). Measurements such as the range of $\delta^{13}C$ observed within the

invertebrate community are useful indicators of the number of trophic resources supporting the community and how much it is relying on the individual resources. On the other hand, the range in $\delta^{15}N$ represents a proxy for the number of trophic transfers occurring within the community (Layman et al. 2007; Jackson et al. 2011). Niche indices have been successfully used to show how resource alteration can alleviate or increase competition within a community and show community reliance on resources (Leberfinger et al. 2011; Serrano-Grejalva et al. 2011). Through experimental manipulation, Syväranta et al. (2010) demonstrated that niche structure and size within a lake community can alter the flow of nitrogen through the system. Although these methods provide great potential for understanding the relationships between nitrogen-cycling and lotic communities, several studies point out that caution should be taken with such approaches highlighting various potential floors (Flaherty and Ben-David 2010; Hoeinghaus and Zeung 2008). A lack of sound background information about species traits and basal resource isotopic signatures can lead to erroneously link changes in isotopic niches with changes in the actual community trophic niche. An example demonstrated that isotopic niche measurements can give opposite conclusion to niche measurements based on morphological traits and gut analysis when insufficient information was know about the test organisms Flaherty and Ben-David 2010). It is acknowledged that these measurements are a developing methodology and many of the issues raised are being addressed within the ongoing methodological development; however currently, validating findings with other measurements is recommended (Jackson et al. 2011).

2.10 Conclusion

Land-use disturbances can cause drastic changes in the cycling of nitrogen within-stream ecosystems. Depending on the way in which land-use disturbances are contributing to the catchment nitrogen cycle a large variety of ecosystem responses can be observed. Thus in order to properly

understand and mitigate these disturbances, an integrated understanding of the nitrogen cycle components is vital. Recent advancements in nitrate stable isotope analyses ($\delta^{18}O$ & $\delta^{15}N$) and the interpretation of stable isotopic foodweb data (mixing models and niche indices) present promising tools for tackling this issue. However, the use of stable isotopes in nitrogen cycling studies is still a developing discipline with a considerable degree of uncertainty primarily regarding assumed fractionation constants. As more controlled experiments and studies investigating the mechanisms controlling fractionation are conducted, much of this variability will be able to be accounted for. In the mean time, using stable isotopic data in conjunction with other environmental and ecological information has and will continue to provide significant insight into the causes and effects of nitrate pollution in freshwater systems.

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Chapter 3: Natural history of the Banks Peninsula and current environmental characteristics

3.1 Geophysical setting

The Banks Peninsula is the eroded remains of a late Tertiary volcanic complex on the east coast of New Zealand's South Island (fig. 3.1). Initially it was an island; however through the progradation of the Canterbury Plains, eastwards from the Southern Alps, it has been intermittently attached to the mainland since the late Quaternary (Soons et al. 2002). During glacial periods wind-deposited loess derived from the Southern Alps has accumulated intermittently on the slopes of the eroded basaltic volcanoes (Wilson 1994; Eikaas et al. 2005). Average elevation is 300 m – 700 m ASL (with Mt Herbert forming the highest point at 920 m ASL), while rainfall ranges from between 600 mm per year to approximately 2000 mm per year at the heads of the southeastern valleys (Soons et al. 2002). This volcanic landscape, in a coastal setting, gives rise to the distinct lotic hydrology observed on the peninsula. Streams typically radiate out from the summits towards the sea and are generally third order rivers at the river's mouth. Catchments are characteristically steep sided with coarse boulder substrate. There are over 100 distinct catchments within the 1102 km^2 region (Harding 2003).

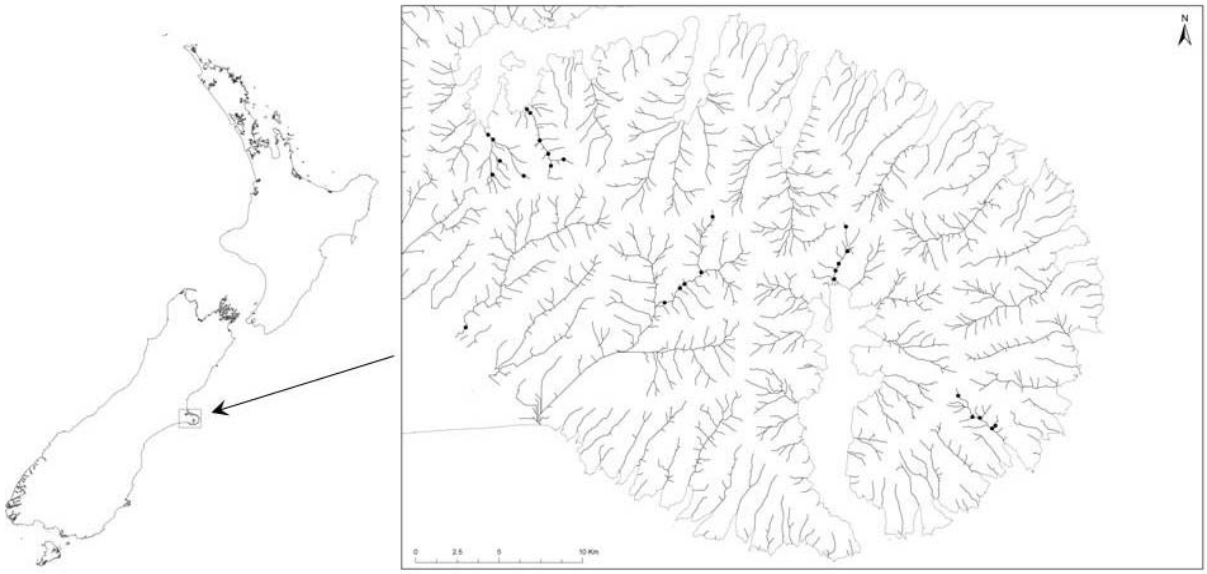

Figure 3.1: Banks Peninsula and associated stream networks. Black dots denote paired water quality and invertebrate sampling sites from summer (2009/2010) and spring 2010, indicating the geographical relationship between the various catchments and sites within catch

3.2 Biota

3.2.1 Terrestrial flora

Prior to human arrival, approximately 1000 years ago, the Banks Peninsula was covered largely in podocarp forest with isolated pockets of beech forests occupying some of the drier eastern and harsher high elevation areas (Soons et al. 2002; Harding 2003). In two distinct phases, the Banks Peninsula underwent extensive landscape modification. The first phase, on the arrival of Polynesians, was primarily forest clearing by fire (Soons et al. 2002). By the time of European settlement, roughly a third of the forest had been cleared in the eastern areas by Polynesians. From 1860 the area was subjected to heavy deforestation through both logging and burning. By 1900 less than 0.001% of the estimated 1840

forest cover remained (Harding 2003). Over this period more than one fifth of the avifauna and unknown number of invertebrates, amphibians and lizards went extinct (Soons et al. 2002). Soil records indicate the Banks Peninsula was occupied by several seabird nesting colonies before the introduction of mammalian predators (Hawke 2003).

Because of the unique climatic conditions, geology, geomorphology and vegetation cover the Banks Peninsula is considered a distinct ecoregion of the South Island by both terrestrial and lotic classifications (Wilson 1994; Harding and Winterbourn 1997). In vegetative terms, the contemporary Banks Peninsula is a heterogeneous landscape dominated by dry stock agriculture (fig. 3.2). Agriculture in the area is associated primarily with cocksfoot and ryegrasses (Harding 2003). There are also substantial patches of land invaded by gorse and broom; a variety of horticultural practices; dairy farming. Land over 300 m a.s.l. is dominated by tussock while on the western flanks, there is substantial *Pinus radiata* production forestry (as denoted by the grey areas in fig. 3.2). There are 400 farms supporting 357,109 sheep and 31,658 cattle over the 80,000 ha of grassland on the peninsula, giving an average stocking ratio of 5.26 stock-units per ha (Ogilvie 2007).

Dairy farming was the Banks Peninsula's primary industry from the mid-1800's through till the 1930's, with numerous creameries established within the various bays and a peak dairy herd of approximately 10'000. However socioeconomic affects of the 1930's depression in New Zealand made these practices uneconomical, resulting in drastically reduced land productivity at which point much of these farms were converted to dry stock (Ogilvie 2007).

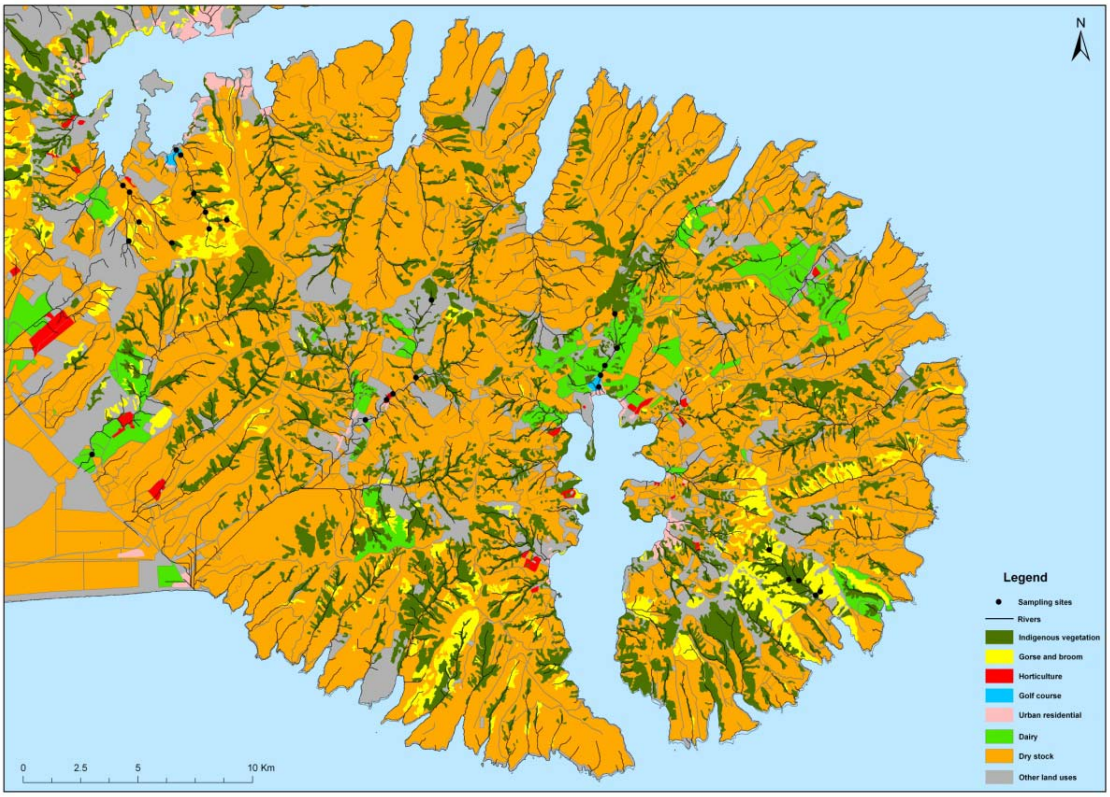

Figure 3.2: Banks Peninsula land-use cover. Black dots denote paired water quality and invertebrate sampling sites from summer (2009/2010) and spring 2010. The land-uses are categorized specifically to the purposes of this study with the unclassified land-uses being denoted by the grey areas

Gorse and broom were recognized as both vigorous and invasive pests on the Banks Peninsula once land was cleared for agriculture in the 1800's. Since then farmers have worked hard to remove gorse and broom from their pasture land (Wilson 1994). While their impact on agriculture have long been recognised, only recently have their impact on native ecology been investigated. It has since been shown to be a capable nursery crop for regenerating native forest (Wilson 1994) and also a refuge habitat for

endemic terrestrial invertebrates (Harris et al. 2004). Little is known on its impacts on adjacent lotic systems however.

3.2.2 Lotic fauna

Previous research has shown that the most common lotic macroinvertebrate orders on the Banks Peninsula were Deleatidium, Coloburiscus, Macropelopiini, Potomypurgus and Eukiferiella (Harding et al. 1997). These orders are also all found in-streams in other South Island ecoregions; however there are also several species know to be endemic to the Banks Peninsula. When comparing functional feeding groups, the Banks Peninsula is over represented in abundances of filter feeders relative to other South Island ecoregions (Harding et al. 1997). Macroinvertebrate communities between forested and pasture reaches have been shown to differ significantly (Harding 2003) with higher ETP richness and several regional endemics restricted to forested reaches. Several fish species are also know to be present in Banks Peninsula streams. Long finned eels are the most common fish species followed by short finned eel, bully species and several galaxid species (Ogilvie 2007). The presence of at least one galaxid species (*Galaxias brevipennis*) is known to be influenced by forest cover. Whilst little is known of the distribution of other fish species, it is presumed that eels and bullies have a wider distribution (Eikaas et al. 2005).

3.3 Catchment summaries

3.3.1 Hukaika Catchment

The Hukaika is a sub catchment of the greater Little River system which feeds into Lake Forsyth, a shallow coastal lake on the southern side of the Banks Peninsula. The catchment begins by flowing out of Fitzgeralds reserve, a remnant/regenerating native bush area before flowing through primarily low density dry stock agriculture. This land is by in large leased out pasture with transient stock (fig. 3.3); however for the duration of this study, it was predominantly dry stock sheep and beef being run (Young

pers. comm.) Whilst no riparian fencing was observed down the length of the river, the steep nature of the catchment meant that large portions of the stream's riparian margin were inhabited with willow and mixed native species which excluded stock. The lower reaches of the catchment flow through the rural settlement of Cooptown which has several horticultural operations. The orchards consist primarily of stone fruits (approximately 70%) with the remaining area devoted to cherries and market vegetables (Everidge *Pers. comm.*). The largest nursery in the area specializes in rhododendrons with native plants forming a subsidairy.

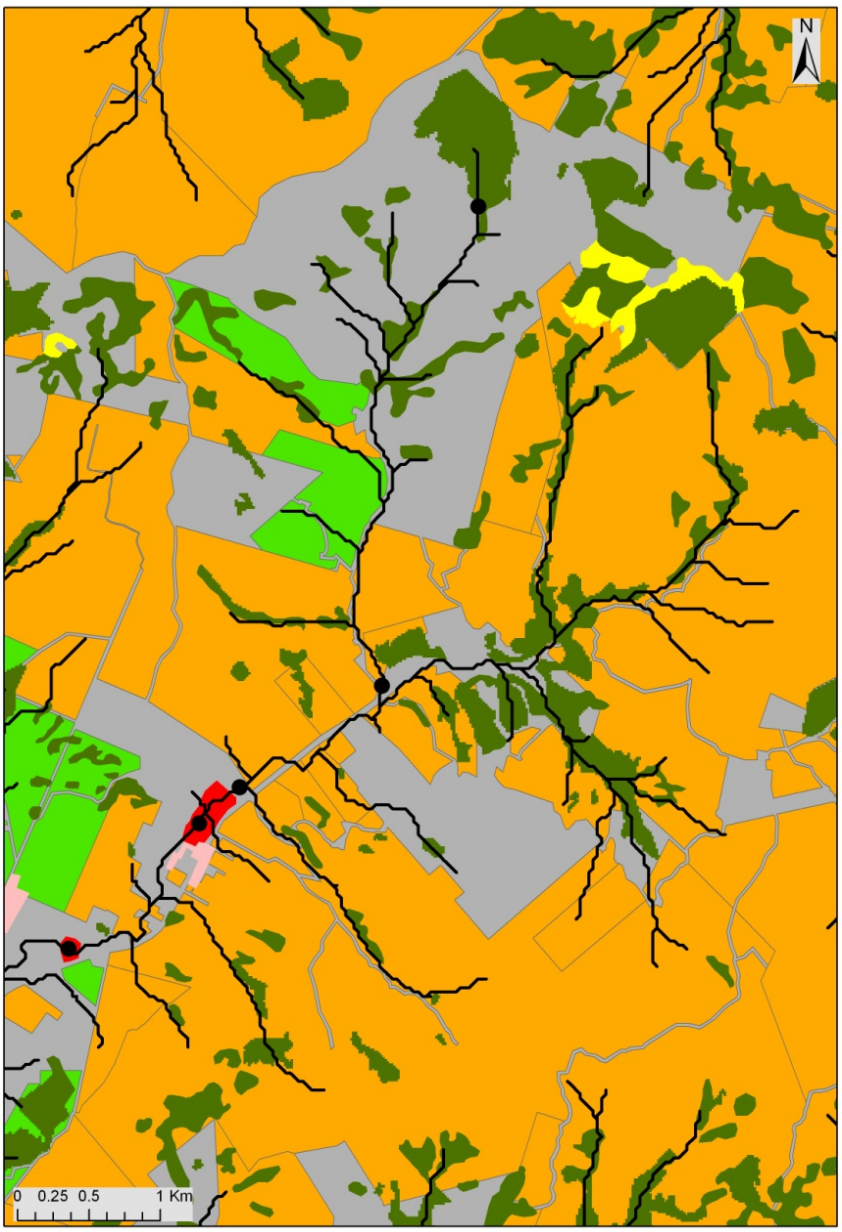

Figure 3.3: Hukaika catchment land-use: Black dots denote paired water quality and invertebrate sampling sites from summer (2009/2010) and spring 2010. The unclassified grey areas within the upper reaches predominantly represent leased pasture in which the animals grazed there is unspecified and transient

3.3.2 Nareby Catchment

The vast majority of the Narbey catchment was purchased by the Maurice White Native Forest Trust in September 1987. Prior to this the land had been a working sheep and beef farm which had become uneconomical to run due to the cost of controlling invasion of gorse onto the farmland. At this stage, the land was retired to native forest known as the Hinewai Reserve. Hinewai reserve is notable in that it adopted a passive approach to control of gorse and broom invasion, instead opting leaving it as a nursery crop for the native seedlings (Wilson 1994). The theory is that the native vegetation will grow up and eventually shade-out the gosre/broom crop. After seven years 30% of the reserve was covered in closed-canopy secondary growth native regeneration forest. However, this native cover has now substantially increased over 20 years (fig. 3.4) and observations show that the gorse/broom is in fact being grown over by secondary native growth (Wilson *pers. Comm..*). Within the lower reaches of the catchment a working farm is still being operated running sheep and beef cattle.

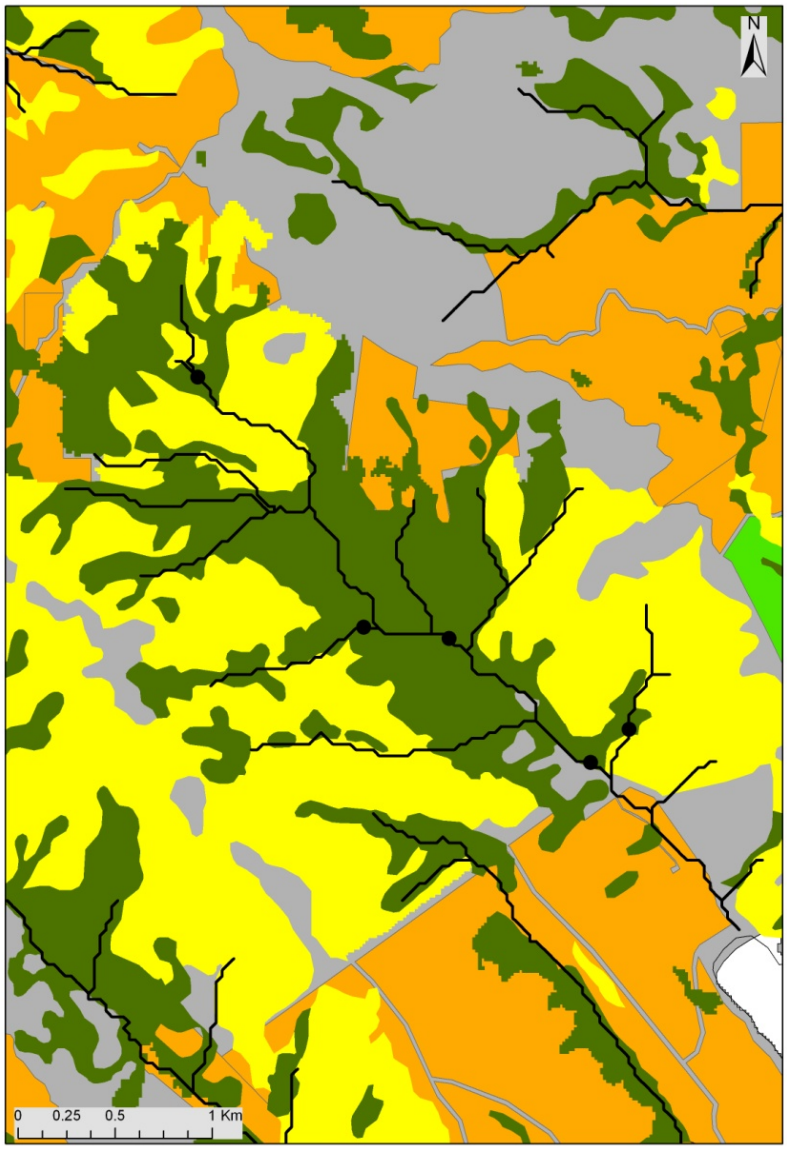

Figure 3.4: Narbey Catchment land-use. Black dots denote paired water quality and invertebrate sampling sites from summer (2009/2010) and spring 2010

3.3.3 Pawsons Catchment

Pawsons Valley was purchased by Jonas Pawson in 1858 which started a legacy of timber milling and dairy farming. The Pawsons Valley has since held a reputation of being a productive dairying area with the herds surviving several trends towards dry stock agriculture (Ogilvie 2007). The settlement of Duvauchelle was established at the foot of the Pawsons Valley in 1843 and is today considered a rural settlement. The lower reaches of the Pawsons stream run through a 18 hole golf course while the majority of up upper catchment is dairy pasture (fig. 3.5). The golf course is an intensively managed system with ammonium seaweed extract fertilizer being applied to the greens every three to four weeks and herbicide weed control along the boulder-packed riparian margins. A general fertilizer (containing phosphate, ammonium, nitrate, potash and other micro-nutrients) is applied to the greens twice a year and annually to the fairways (Miller *pers. comm*..). There are three greens within 20 m of the river channel. At the head of the catchment the stream originates from a patch of regenerating forest which is not excluded from grazing sheep and beef cattle.

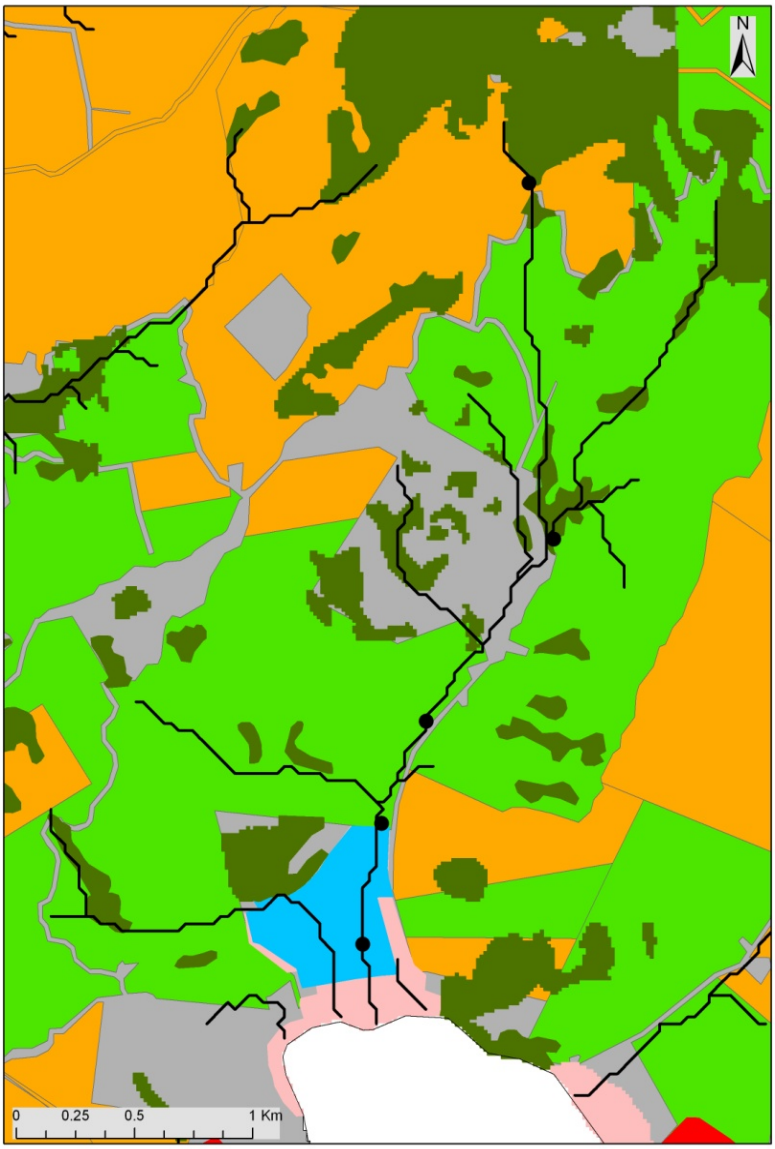

Figure 3.5: Pawsons Catchment land-use. Black dots denote paired water quality and invertebrate sampling sites from summer (2009/2010) and spring 2010. The unspecified grey area at the mid catchment level represents land which is currently leased by an adjacent farmer. Horses and cattle were being grazed here in low densities during the period of the study

3.3.4 Te Wharau Catchment

The Te Wharau catchment is in its entirety contained within Orton Bradley Park. Orton Bradley Park is a private reserve which is owned by the Orton Bradley Trust. The majority of the park land is run as a working farm with low density sheep and beef; however there are also significant areas of gorse and broom and native regenerating forest (fig. 3.6). During the period this study was conducted a large riparian fencing project was underway. This resulted in summer samples being collected from an unfenced site while spring samples, collected from site, had had stock excluded from the riparian margin in the previous 3 months. There are also substantial areas of both remnant and regenerating native forest; an exotic garden containing deciduous trees and an extensive array of Rhododendron species; a 18 hole golf course. The park was established in 1972 with the mantra of being a commons ground. The land on which the park now sits was first settled by Europeans in 1852. Over the next four decades the native forest was progressively cleared in order to establish market gardens and a dairy farm. In 1892 Orton Bradley took over the land and it was it this point that the park setting in the lower reaches of the catchment was established (http://www.ortonbradley.co.nz/park-history.html accessed 24/10/2011). At this same time farming became primarily dry stock and areas of native forest were protected and allowed to re-establish on the recently cleared farmland. As shown by fig. 3.6, substantial areas of this cleared land were also invaded by gorse and broom once left fellow. The golf course at the lowest reaches of the catchment is a 9 hole course which the river meanders through. It is a moderately intensively managed system with a general fertilizer (containing phosphate, ammonium, nitrate, potash and other micro-nutrients) applied to the greens twice a annually and intermittently on the fairways (Cotton *pers. comm*.). Generally the riparian margins are passively managed with tussock grasses and

patches of mixed mature exotic and native understory growth occupying several reaches. There are two greens within 20 m of the river channel.

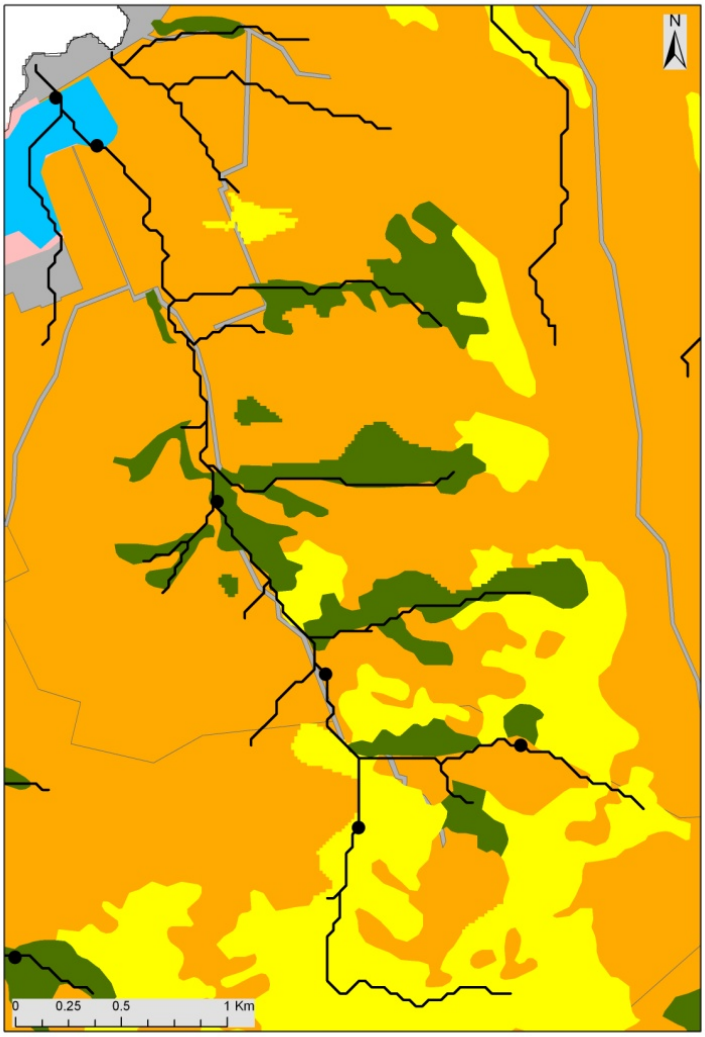

Figure 3.6: Te Wharau Catchment land-use. Black dots denote paired water quality and invertebrate sampling sites from summer (2009/2010) and spring 2010

3.3.5 Waiake Catchment

The Waiake is the most laterally expansive of the catchments surveyed in this study (fig. 3.7). The catchment is primarily occupied by two privately owned working farms; Louden farm on the western side of the catchment and Manor Farm on the eastern side. Both farms run sheep and beef cattle in low densities. The highest point of the catchment is an area of secondary native growth and native scrub on the lower edges of Mt Bradley. Several of the steep first order valleys within the catchment have been aggressively invaded with gorse and broom (fig. 3.7) which has been left there for at least 10 years (King *pers. comm.*.).

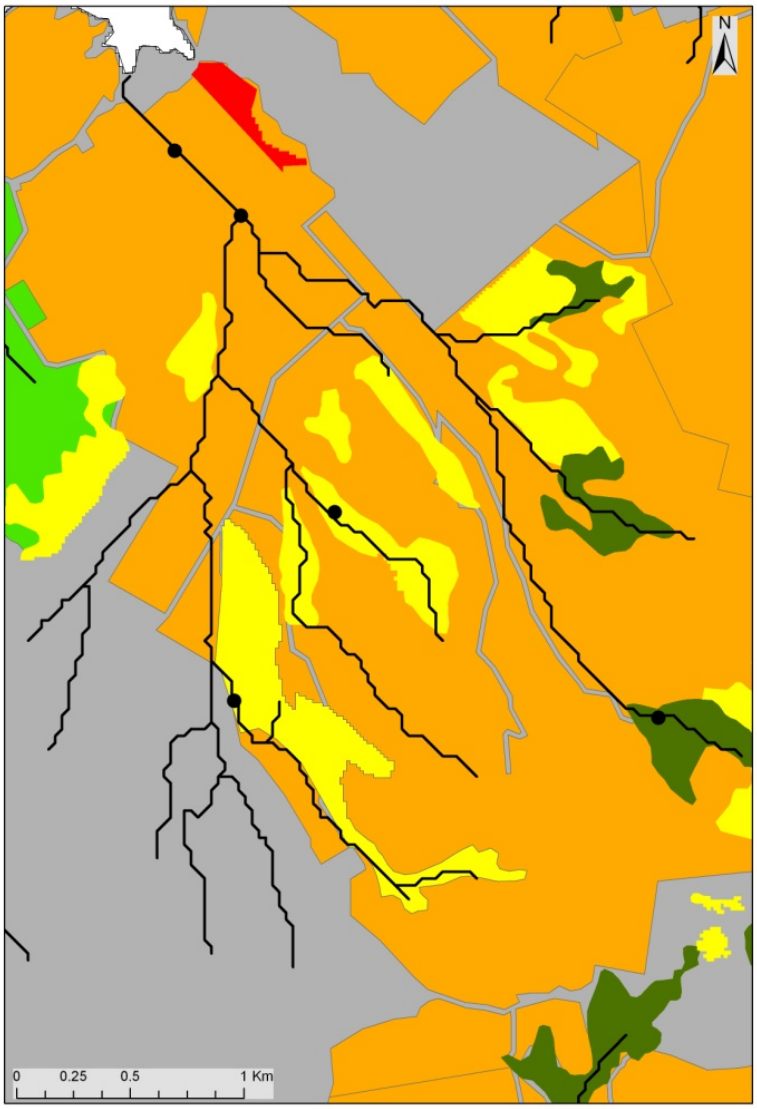

Figure 3.7: Waiake Catchment land-use. Black dots denote paired water quality and invertebrate sampling sites from summer (2009/2010) and Spring 2010. The unspecified grey areas within this catchment are occupied by production Pinus radiata forestry

3.4 Literature cited

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Chapter 4: Using nitrate stable isotopes to investigate land-use impacts on water chemistry within-streams on the Banks Peninsula New Zealand

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4.1 Abstract

Understanding the relationship between land-use and stream nitrate levels is a primary focus of environmental managers due to the acute environmental and health risks posed by excess nitrate. However, as the majority of most catchments' nitrogen budgets are coming from diffuse sources the relationship can be blurry; while significant nitrate sources could potentially be missed. Nitrate $\delta^{15}N$ and δ18O stable isotopic values provide a powerful analytical tool for investigating the relationships between land-use and stream nitrate. Here I compare relationships between land-use and nitrate δ15N and δ18O values, as well as a suite of environmental physicochemical parameters, within-streams on the Banks Peninsula New Zealand. Of the six land-uses investigated (indigenous regenerating forest, horticultural, golf course, dry stock agriculture, dairy agriculture and land invaded by exotic N-fixing shrubs (gorse and broom)) gorse and broom streams had significantly the highest nitrate concentrations ($P < 0.001$). Stable isotopic data indicated that this nitrate in these streams was in fact being fixed by the shrubs rather than being a land-use legacy effect. Further, comparing the patternns seen in nitrate $\delta^{15}N$ and δ18O values between land-uses showed that all land-uses other than regenerating forest displayed

significant positive linear nitrate $\delta^{15}N - \delta^{18}O$ fractionation relationships possibly due to nitrate uptake. However, the physicochemical controls on observed nitrate fractionation varied significantly between land-uses. This study identifies a substantial and novel nitrate source at the landscape level while also highlighting land-use specific differences in the controls of in-stream nitrate removal. These findings should be of interest to researchers and managers both nationally and internationally.

4.2 Introduction

Understanding the sources of nitrate pollution is one of the most pressing issues faced by freshwater managers (Erimsam et al. 2008; Nestler et al. 2011). Over the last century, it is estimated that the terrestrial export of reactive nitrogen (i.e. nitrogen that is available organisms) has increased by 60 – 80% globally; while this is predicted to increase by a further 20 – 40% over the next 50 years (Galloway et al. 2004; MEA 2005). The environmental and socio-economic impacts associated with this increased freshwater nitrogen loading are both significantly detrimental and wide ranging (Carpenter et al. 1998; Gruber and Galloway 2008). The dominant form of nitrogen pollution within freshwater systems is nitrate (Xue et al. 2009). Nitrate additions cause eutrophication (Dodds 2007); reduce and alter vital ecosystem services (Benstead et al. 2009); lead to changes in community structure (Carpenter et al. 1998; Davis et al. 2009) and ultimately result in freshwater species and habitat losses (Singer and Batin 2007; Anton et al. 2011). Globally, nitrogen pollution is one of the primary drivers of ecosystem change (Vitousek et al. 1997; Galloway et al. 2008). These environmental impacts can also have significant economic flow on effects. As an example, in New Zealand the environmentally friendly image created through the "100% Pure" national branding campaign is estimated to have added over $8 billion (>85%) to the tourism industry (Bertrum 2010). Anecdotally, this eco-branding has been linked to the significant increases in international exports of food and beverages as well as manufactured goods (Pratt

and Pratt 2008). Much of this brand's image is based on the ecological health and integrity of New Zealand's waterways. The Rotorua lakes in New Zealand's central North Island, a tourism hotspot, are an example of waterways which have become impaired by eutrophication; this has resulted in the loss of important fisheries, access to drinking water and access to the waterways for recreational and customary purposes. In response to this, central government and the regional council committed $144.2 million to aid the restoration of these lakes (www.ebop.govt.nz accessed 26/09/2011).

More concerning than the fiscal costs, is the risk of human infant mortality through chronic nitrate toxicity. When nitrate concentrations in drinking water supplies exceed 11.3 ppm, infants consuming the water are at risk of methemoglobinemia (blue baby syndrome) as nitrate binds to the red blood cells in place of oxygen thus suffocating the victim (Gupta et al. 2008). Controlling nitrate concentrations in waterways is, thus, an imperative environmental, social, cultural, and human health objective.

One of the main obstacles to achieving environmental restoration goals and reducing nitrate concentration is understanding where the primary sources of nitrate are within a landscape (Dodds 2007; Xue et al. 2009; Nestler et al. 2011). Moreover, nutrients are cycled at an ecosystem level. This means that streams are tightly coupled with their catchments and will be impacted by disturbances occurring at multiple stages of the nutrient cycle (Galloway et al. 2008). This is especially important given the finite resources available to environmental managers (Nestler et al. 2011). Many modern catchments contain a variety of land-uses, all potentially contributing to the waterway's nitrate budget and therefore making identifying sources difficult (Dodds and Oakes 2007; Weijters et al. 2009). When combined with other lines of evidence nitrate $\delta^{15}N$ and $\delta^{18}O$ stable isotopic analysis provides the most promising method available for identifying sources of nitrate (Xue et al. 2009).

Stable isotopic analysis of nitrate is based on the premise that specific physical and biological stages within the nitrogen cycle leave distinguishable isotopic imprints on the nitrogen and/or oxygen atoms within a population of nitrate molecules. Dual stable isotopic analysis of nitrate has been successfully used in field studies to identify the primary sources of nitrate in both contaminated and pristine environments as well as identifying physical and biological processes which are impacting the system's nitrogen cycling (Kendall et al 2007; Xue et al. 2009). The field of stable isotopic nitrate source tracking has been developing for almost two decades. While significant advances in the understanding of the controls on nitrate stable isotopic values have been achieved during this time, the consensus is that there are still important gaps within the field (Kendall et al 2007; Xue et al. 2009; Kool et al. 2011; Nestler et al. 2011). One such gap is the immense imbalance in research conducted in the northern and southern hemispheres (Bowen et al. 2009; Xue et al. 2010). One of the primary goals of the Isoscapes project, which aims improve our understanding of global stable isotopic biogeochemical cycles, has highlighted the lack of data outside of North America and Europe as a significant hindrance to our understanding of biogeochemical patternns and relationships. The majority of research to date has been conducted in perennial temperate rivers in Northern America and Europe (Bowen et al. 2009). Extrapolating these findings to other regions is somewhat tenuous; such questions of generality have already been raised in regards to other aspects of lotic ecology (Winterbourn et al. 1981). To properly understanding the relationship between land-use, nitrate concentration and nitrate isotopic values, research needs to be extended to other parts of the world.

The Banks Peninsula on the east coast of New Zealand's South Island represents a unique area to investigate the relationship between land-use, nitrate concentration and nitrate isotopic values. The Banks Peninsula is characterised by short, steep catchments, often over exposed volcanic bedrock and a

simplified hydrologic system with discrete catchments (Wilson 1994; Harding 2003). Generally these catchments also contain multiple land-uses (Harding 2003). Simple hydrology allows for a relationship between land-use and stream water chemistry to be more easily identified (Xue et al. 2009). Moreover, within a relatively limited geographical area, the Banks Peninsula has a wide variety of land-uses. These include; remnant and regenerating indigenous forest, low intensity sheep and beef agriculture, high intensity dairy agriculture, horticulture, golf courses, and fallow land invaded by exotic nitrogen fixing shrubs gorse (*Ulex europaeus*) and scotch broom (*Cytisus scoparius*). A potentially important vegetation on the Banks Peninsula are exotic N-fixers (gorse and broom) which may be significant sources of nitrate. This combination of six land-uses which the Banks Peninsula provides offers an exceptional opportunity to investigate the relationship between catchment land-use practices and water quality.

This chapter investigates the relationship between land-use, nitrate concentration and nitrate isotopic signature within-streams on the Banks Peninsula. Specifically I will address 1) which land-uses are associated with the highest nitrate concentrations; 2) what isotopic values of nitrate are associated with specific land-uses; 3) the mechanisms within the nitrogen cycle which control these isotopic values. While having direct and important implications for the management of waterways both in New Zealand and internationally, this research will provide valuable insight into how an important ecosystem function (nitrogen cycling) responds to a perturbation (nitrogen loading) across 5 different contemporary land-uses compared with nitrogen cycling in a near pristine system.

4.3 Methods

Sampling was conducted over 2010 during summer, autumn and spring over the six land-use classes (table 4.1). The summer and spring sampling consisted of collecting water samples and organic ecosystem components for stable isotopic analyses ($\delta^{13}C$ & $\delta^{15}N$ for organic samples and $\delta^{18}O$ & $\delta^{15}N$ for

NO_3 samples). The autumn field campaign focused solely on collecting physicochemical measurements and water samples within the study catchments.

Table 4.1: summary of study design. Land-uses are compared by riparian canopy cover, the presence of grazing stock (sheep and cattle) and fertilizer application. Fertilizer application rates were based on anecdotal information from landowners: absent) no known fertilizer application, infrequent) no fertilizer applied during the field sampling period, present) fertilizer applied during field sampling period

Land-use	n	Canopy cover	Stock	Fertilizer
Indigenous regeneration	22	Closed	Absent	Absent
Gorse and broom	10	Closed	Absent	Absent
Horticulture	4	Open	Absent	Infrequent
Golf Course	4	Open	Absent	Present
Dry stock agriculture	16	Open	Present	Infrequent
Dairy agriculture	8	Open	Present	Present

4.3.1 Study area

Banks Peninsula is the eroded remains of a late Tertiary volcanic complex on the east coast of New Zealand's South Island (fig. 4.1). Initially it was an island; however through the progradation of the Canterbury Plains, eastwards from the Southern Alps, it has been intermittently attached to the mainland since the late Quaternary (Soons et al. 2002). During glacial periods wind-deposited loess derived from the Southern Alps have accumulated on the slopes of the eroded basaltic volcanoes (Wilson 1994; Eikaas et al. 2005). Average elevation is 300m – 700m ASL (with Mt Herbert forming the highest point at 920 m ASL), while rainfall ranges from between 600 mm per year to approximately 2000 mm per year at the heads of the south-eastern valleys (Soons et al. 2002). Streams typically radiate out from the summits towards the sea and are generally third order at the river's mouth. Catchments are

characteristically steep sided with coarse boulder substrate. There are over 100 distinct catchments within the 1102 km^2 region (Harding 2003).

Prior to human arrival, approximately 1000 years ago, the Banks Peninsula was covered largely in podocarp forest with isolated pockets of beech forests occupying some of the drier eastern and harsher high elevation areas (Soons et al. 2002; Harding 2003). However, anthropogenic deforestation, particularly since the arrival of European settlers in the mid-1800's, has drastically reduced the forest cover through both logging and burning. By 1900 less than 0.001% of the estimated 1840 forest cover remained (Harding 2003). Indigenous forest is now largely restricted to small stream valleys; from here is regenerating outwards (Wilson 1994; Harding 2003). Today, the primary land-use is low intensity dry stock agriculture (e.g., sheep). However, there are also significant areas of dairying and horticulture. Gorse and broom vegetation invaded large areas and there are several small towns and golf courses. While the area has a rich tradition of dairy farming, much of this was converted to low intensity dry-stock farming over the last half century (Ogilvie 2007).

4.3.2 Land-use classifications

Land-use classifications were initially based on GIS land cover and agricultural practice databases (*Ministry for the Environment database 2009*). Selected sites were then ground-truthed through field reconnaissance. Distribution of each land-use within the six study catchments is displayed in figure 4.1.

Indigenous regeneration

Indigenous vegetation regenerations sites were considered areas of secondary indigenous growth or more mature (Podocarpus species > 100 years old). The dominant secondary growth vegetation is Kanaka (*Kunzea ericoides*) as well as occasional broadleaf (*Griselinia latoralis*) and *Podocarpus* species. This is commonly associated with an understory of largely *Coprosma* and *Dracophyllum* species, five

finger *(Pseudopanax aboreus*) as well as a variety of other sub-canopy species common to New Zealand Indigenous forest. A variety of fern species commonly form the ground cover (Wilson 1994; *pers. obers.*).

Gorse (Ulex europaeus) and broom (Cytisus scoparius)

Gorse and broom are functionally interchangeable in terms of their growth, shade tolerance and N-fixing (Drake 2011; Magesan *in press*); thus were considered a single classification for this study as is consistent with land cover information. Areas of gorse and broom were defined by the Land Cover Data Base (Ministry for the Environment 2009) as those in which they were the dominant vegetation cover (i.e. >60%). When areas are invaded by gorse and broom, it will form a dense canopy forming a monoculture with a humus groundcover (Wilson 1994; Magesan *in press*).

Horticulture

Horticultural land consisted of managed perennial commercial trees. Of the two sites sampled, one was a stone fruit orchard comprised primarily of peach, apricot and cherry trees. The other site was a nursery which specialised in *Rhododendron* cultivars with a small standing of New Zealand indigenous shrubs as well.

Golf course

Two golf courses were also included as these might represent areas of high human fertiliser additions which could influence nearby streams.. The golf courses consisted of extensive areas of intensively managed exotic grassland with small areas of (primarily exotic) trees. Both courses had similar greens management practices (Cotton pers. comm.; Miller pers. comm.). The primary fertiliser

applied in these systems was Country Club 18-1.3-15 by Lebanon products. It contained ammonium-nitrate (7.4%), water soluble methylene urea (6.8%) and organic nitrogen compounds (6.5%).

Dry stock agriculture

Dry stock agriculture is used to describe stock which are not milked. Primarily this consists of beef cattle and sheep. Dry stock agriculture is generally considered low intensity land management because of the characteristically low stocking rates. The average stocking rate for the Banks Peninsula is 7.0 stock-units per ha (Ogilvie 2007).

Dairy agriculture

Dairy farming in New Zealand is primarily on intensively managed exotic grassland. Socking rates are increasing with approximately 3.2 dairy cattle per hectare (25.5 Stock-units/ha) (Clark et al. 2009). Herds are primarily made up from Jersey and Frisian breeds and milked at a centralised milking shed twice daily.

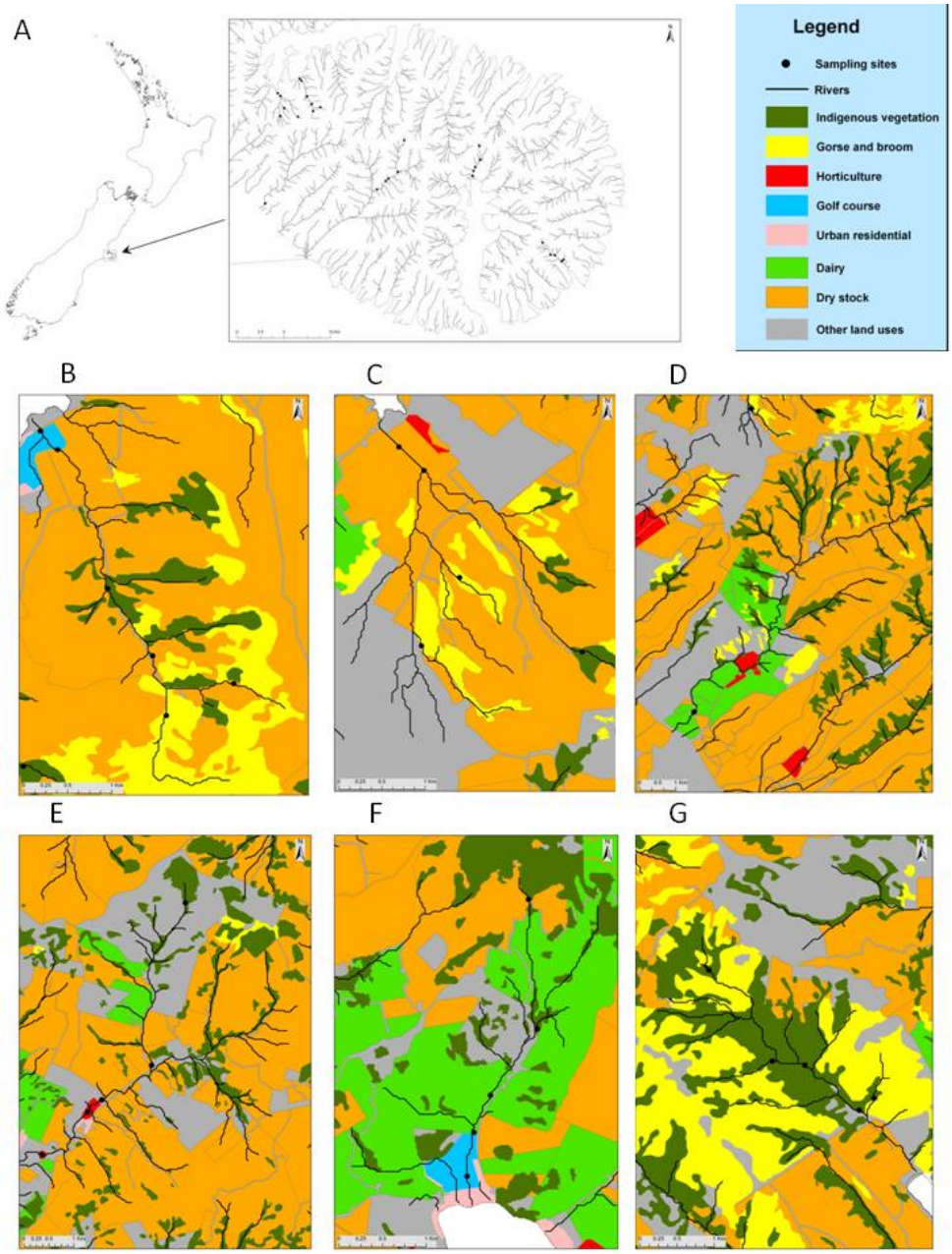

Figure 4.1: Study sites on the Banks Peninsula and land-use within the study catchments. All study sites within the six catchments are shown. The catchments are: B) Te Wharau (n = 9), C) Waiake (n = 8), D) Kaituna (n = 1), E) Hukaika (n = 8), E) Pawsons (n= 8), F) Narbey (n = 8). Grey areas represent a mosaic of land-uses which were not investigated in this study; primarily this consisted of leased out grazing land unspecified lifestyle properties and production forestry.

4.3.3 Field data collection

In the field, spot measurements of in-stream physicochemical parameters were taken (table 4.2). Water samples were filtered in the field using 45 µm GFF filters for subsequent ion concentration analysis, nitrate – $\delta^{18}O$ & $\delta^{15}N$ analyses and water – $\delta^{18}O$ & $\delta^{2}D$ analyses. In total 16 parameters were recorded for each site providing a suite of measurements for the physical and chemical condition of both the riparian and in-stream habitats (table 4.2). Filtered water was stored in the dark and on ice in pre-rinsed polyethanol containers

Table 4.2: suite of parameters recorded at each sampling site indicating units in which they are presented throughout the paper and data collection method. *canopy cover was based on methods described within Harding et al. (2009)

Parameter	Units	Method
Nitrate – $\delta^{15}N$	‰ (Air)	Bacterial denitrification
Nitrate – $\delta^{18}O$	‰ (VSMOW)	Bacterial denitrification
Nitrate-N	Parts per million (ppm)	Colormitric spectrotography
Nitrite-N	Parts per billion (ppb)	Colormitric spectrotography
Ammonium-N	Parts per billion (ppb)	Colormitric spectrotography
Phosphate-P	Parts per billion (ppb)	Colormitric spectrotography
Water – $\delta^{18}O$	‰ (VSMOW)	TCEA mass spectrometry
Water – $\delta^{2}D$	‰ (VSMOW)	TCEA mass spectrometry
Soil C:N*	C:N ratio	EA mass spectrometry
Soil – $\delta^{15}N$*	‰ (Air)	EA mass spectrometry
Dissolved Oxygen	mg/L	In-situ probe
Conductivity	Micro-Siemens (µS)	In-situ probe
pH	Proportion H ions	In-situ probe
Temperature	Degrees Celsius (°C)	In-situ probe
Canopy cover*	Ranked	Percentage light penetration scorings*
Longitudinal distance	Meters (m) from hydrologic source	GIS hydrology maps

Soil samples were collected using a trowel taking < top 5 cm of soil as consistent with other studies analyzing soil isotopic values (Stevenson et al. 2010). Soil was taken from the riparian margin within 5 m of the active channel. Samples were bagged and stored in the dark on ice until returning to the laboratory at which stage it was transferred to a freezer (-20°C) until further analyses.

4.3.4 Laboratory processing

In the laboratory soil samples were dried in the oven after which any root material present was removed from the soil matter. Soil samples were then homogenized using a mortar and pestle then passed through a sieve to exclude coarse (>0.1mm) inorganic sediment being incorporated into the final sample. Soil samples were analyzed for $\delta^{13}C$ & $\delta^{15}N$ and C:N ratio using an elemental analyzer attached to a Delta V plus Thermo-Finnegan isotope ratio mass spectrometer at the University of Canterbury Stable Isotope Laboratory. All analyses were compared against international standards Pee Dee Belemnite (PDB) for carbon and Air for nitrogen. All measurements were expressed in the standard delta (δ), per-mil (‰) relative to the international standard. Analytical precision for $\delta^{15}N$ was approximately 0.05‰ and 0.01‰ for $\delta^{13}C$.

On returning to the laboratory an aliquot was removed from all water samples before freezing for ion concentration analysis. Remaining sample water was stored frozen until eventual stable isotopic analyses. Summer and autumn samples were measured at the University of Canterbury for concentrations of Nitrate (NO_3^--N), nitrite (NO_2-N), Ammonium (NH_4^+-N) and phosphate (PO_4-P) using the *EasyChem* Automatic sequential Colorimetric analyzer (SYSTEA) following the Lachat method. Units were recorded in parts per billion with an accuracy of 1 ppb or better. Due to disruptions caused by the Christchurch February 22nd earthquake, spring samples were not able to be analyzed in the same laboratory; thus were sent through to a commercial laboratory at the National Institute of Water and

Atmospheric sciences (NIWA) in Hamilton New Zealand. Spring samples were analyzed for nitrate, nitrite and phosphate using a flow injection analyzer following the Lachat method. While this discrepancy between laboratories does create a potential bias within the data, a comparison to historical data (Environment Canterbury *unpublished data*) suggested that our observed nitrate concentrations were not significantly different from previously measured concentrations for sites within three of our five streams ($p = 0.38$) which were able to be compared. Whilst we lacked statistical power to test for a seasonal effect, concentration patternns were comparable to the previous research results with summer concentrations being lower than those of autumn and spring.

4.3.5 Nitrate $\delta^{18}O$ & $\delta^{15}N$ analysis

Nitrate $\delta^{18}O$ & $\delta^{15}N$ values were determined using the bacterial denitrification method (Sigman et al. 2001; Casciotti et al. 2002). Analyses were conducted in the Stable Isotope Tracers Laboratory in the Water Resources Division of the United States Geological Survey, Menlo Park, California USA. While the method is thoroughly detailed within Sigman (2001) and Casciotti (2002) here we will cover, in short, the methodology specific to this study. Batches of nutrient-amended triptic soy broth were inoculated with *Psudomonas aureofiecens*. After approximately seven to ten days of incubation, the culture broth was tested to ensure that no residual nitrate or nitrite remained and that cell density was within expected parameters. The culture broth was then concentrated 10 times through centrifuging before 2 ml aliquots were dispensed into 20 ml glass headspace crimp-top vials (National Scientific) which were sealed with PTFE/white silicone aluminium crimp seals. Vials were then purged with N_2 gas to create an anaerobic environment. At this stage, water samples were added to the anaerobic culture vials and left incubated for 24 hours in order to allow the microbial culture to quantitatively convert all the dissolved nitrate to N_2O gas. Sample and standard volumes were adjusted to ensure that all vials contained the

same initial amount of nitrate, which ranged from 50 to 75 nmols of nitrate depending on the analytical run. Prior to stable isotopic analysis, microbial activity within the vials was terminated through a 0.1 – 0.2 ml addition of 10N NaOH. The vials were loaded into autosampler racks, and the gas in the vials was then introduced via a custom-built autosampler into an IsoPrime continuous flow mass spectrometer. The autosampler purges each vial for 10 minutes to strip all the nitrous oxide from the sample. The gas stream carrying the sample passes through a series of traps to remove water vapor and organic compounds, and is then concentrated in a liquid nitrogen cryotrap. The N_2O is analyzed for both $\delta^{15}N$ and $\delta^{18}O$, and these values are used to calculate the $\delta^{15}N$ and $\delta^{18}O$ of the nitrate in the original sample.

The raw data is corrected for instrument drift, size linearity, blank contribution, and fractionation effects by using repeated analyses of five different standards (international standards USGS-34, USGS-35, and IAEA-N3; internal standards 9707 and WEN-D), a standard containing a blank (vials with media but no added nitrate). All samples are prepared and analyzed in duplicate on the same day, and then analyzed a third or more times until the precisions were acceptable (<1.0 ‰ for nitrate - $\delta^{15}N$, <2‰ for nitrate - $\delta^{18}O$). Average precisions for nitrate - $\delta^{15}N$ & $\delta^{18}O$ for replicate samples are 0.2‰ and 0.7‰ respectively. The $\delta^{15}N$ and $\delta^{18}O$ values are reported in ‰ relative to the Air and VSMOW standards, respectively.

Due to the optimum total amount of nitrate required for analysis with the current mass spectrometer configuration, and injection sample size limitations based on the vials used for the autosampler, a sample nitrate concentration of 20μM or higher was necessary to achieve the best analytical performance. This meant that some of the lower nitrate concentration samples had to be evaporitively concentrated prior to analysis. This involved taking a volume of sample which contained sufficient moles of nitrate (30 μM) and increasing its pH circum 9 using concentrated KOH. The increased

pH immobilized the nitrate within the sample. Once this was achieved, and the initial sample weights were recorded, samples were frozen and placed in a -80°C vacuum sealed freeze-drier. Samples were removed once they had reduced to approximately 20 ml. The target final volume of approximately 20mL was calculated based on the initial available sample volume, initial nitrate concentration, and the ideal final concentration. Since analytical sensitivity is much higher for nitrate - $\delta^{15}N$ in comparison to nitrate - $\delta^{18}O$, some samples which did not contain enough initial nitrate to reach the optimum final concentration could only be analyzed for $\delta^{15}N$. After concentration, the sample pH was lowered using dilute acid (10% 1N HCl) to approximately that prior to KOH addition, they were weighed, their final weight recorded, the new nitrate concentration was calculated, and the samples were shipped off for analysis.

Nitrite has been shown to interfere with recorded nitrate $\delta^{18}O$ & $\delta^{15}N$ values when in excessive concentration (Granger and Sigman 2009; Casciotti et al. 2007). Any samples in which nitrite exceeded 2% of the nitrate body were deemed as having a nitrite concentration that was high enough to significantly increase the probability of producing erroneous $\delta^{18}O$ & $\delta^{15}N$ data. As a result, these samples were treated with sulphamic acid, as described in Granger and Sigman (2009) to drive off nitrite. If samples were to also be concentrated, this nitrite purging step was conducted prior to freeze-drying.

4.3.6 H_2O – $\delta^{18}O$ & $\delta^{2}D$ analysis

Prior to samples being sent away for nitrate isotopic analysis, an aliquot of each sample (approximately 2 ml) was taken for H_2O – $\delta^{18}O$ & $\delta^{2}D$ analysis. Analyses were conducted in the University of Canterbury Stable Isotope Laboratory using a thermal-controlled elemental analyzer attached to a Delta V plus Thermo-Finnegan isotope ratio mass spectrometer. Average precisions for H_2O – $\delta^{18}O$ and

H_2O – δ^2D for replicate samples are 0.2‰ and 0.7‰ respectively. The $\delta^{18}O$ and δ^2D values are reported in ‰ relative to VSMOW standards.

4.3.7 Data analysis

Data were then analysed to test for land-use differences within water chemistry and nitrate isotopic signature data as well as investigating correlative and causal relationships within the measured physicochemical parameters. All data were analysed using R version 2.13.0. Due to the non-orthogonal experimental design, linear mixed effects models were used for all analyses where comparing land-uses. Mixed effects models provide powerful analysis for testing interactions between predictors within non-orthogonal data (Crawley 2007). The study design meant that land-use, as a predictor, was nested within season and longitudinal position within the catchment. When comparing intra-land-use nitrate $\delta^{18}O$ & $\delta^{15}N$ values with environmental parameters, linear regression was used.

4.4 Results

4.4.1 Nitrate concentrations

Nitrate-N concentrations in Banks Peninsula streams were generally low ranging between <0.001 and 2.5 ppm Of the six land-uses investigated within this study, streams within catchments dominated by exotic N-fixing shrubs (gorse and broom) had significantly higher mean nitrate-N concentrations ($P < 0.001$). Gorse and broom catchments had on average 200% higher nitrate concentrations than streams in other land-use catchments (fig. 4.2). Of the physicochemical parameters measured (temperature, pH, conductivity and dissolved oxygen) only dissolved oxygen displayed a significantly positive correlation with nitrate concentrations ($P = 0.013$, $F = 7.53$, $df = 1$).

Analysis also revealed that there was a seasonal effect where all streams had significantly lower nitrate concentrations during the summer than in spring and autumn. There was no downstream longitudinal affect on nitrate-N concentration (P = 0.890).

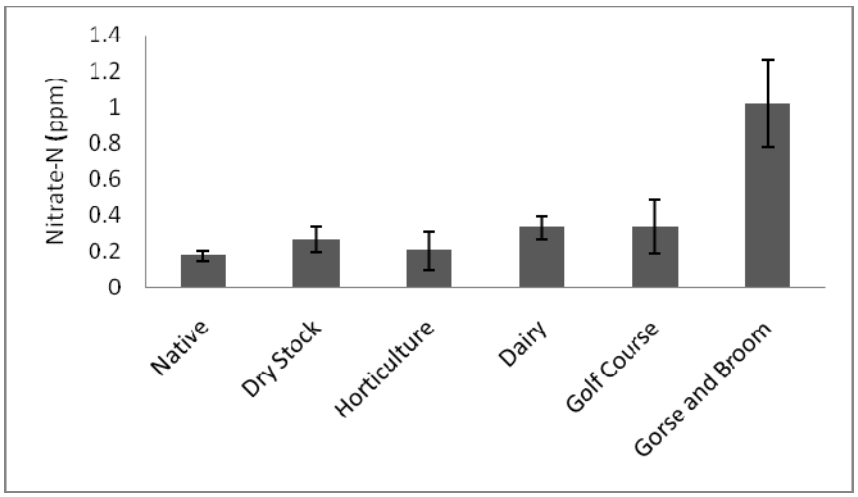

Figure 4.2: Nitrate-N (NO3) concentrations of Banks Peninsula streams compared by land-use. Gorse and broom sites had significantly higher nitrate-N than all other land-uses (P < 0.001, F = 51.3, df = 5). Error bars = 1 SEM

4.4.2 Nitrite-N, ammonium-N and phosphate-P concentrations

There was no significant covariation between any of the measured ions (nitrate, nitrite, ammonia and phosphate) between sites indicating that the reported relationships are independent (P = 0.523). Concentrations of all three of the other measured anions (nitrite, ammonium and phosphate) were all characteristically low across all land-uses except dairy streams in which they were all elevated (fig. 4.3).

While phosphate-P concentrations within dairy streams were on average over 200% higher than those recorded in other land-use streams, there was no significant relationship between land-use and phosphate concentration ($P = 0.4963$, $F = 4.32$, $df = 5$). This lack of significance is likely due the high variation associated phosphate concentrations in dairy stream (fig. 4.4.3). Phosphate concentration was positively correlated with dissolved oxygen ($P = 0.034$, $F = 7.77$, $df = 1$) and temperature ($P < 0.001$, $SS = 10.49$, $df = 1$).

Ammonium-N concentrations showed no significant relationship with land use ($P = 0.210$). There was, however, a significant land-use by season interaction effect ($P = 0.025$, $F = 3.21$, $df = 10$); during the summer dairy streams had higher ammonium concentrations than all other land-uses. No other significant relationships were observed between ammonium and any of the measured in-stream or riparian parameters.

Nitrite-N concentrations in dairy stream were significantly higher than other land-uses ($P = 0.014$, $F = 3.34$, $df = 5$). However none of the measured in-stream or riparian parameters correlated with nitrite concentration within my study streams

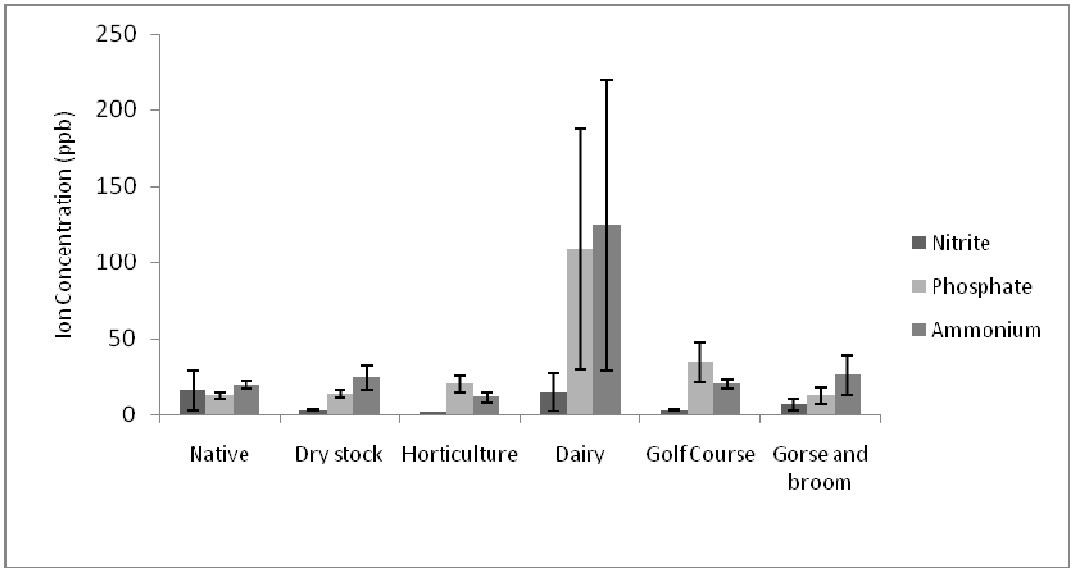

Figure 4.3: mean concentrations of the three secondary ions (nitrite: NO2-N , phosphate PO4-P, and ammonium NH4-N) for stream catchment land-use. Concentrations are displayed in parts per billion (ppb). Error bars = 1 SEM

4.4.3 Nitrate $\delta^{15}N$ and $\delta^{18}O$ values

When compared with global averages, all data points, except for one dairy stream sample, fell within the isotopic range of nitrate derived from the nitrification of soil organic matter, NH4 fertilizer or manure and septic waste (fig. 4.4). Dairy sites on average had the highest nitrate $\delta^{15}N$ and $\delta^{18}O$ values and indigenous regeneration sites had the lowest (table 4.3). Despite the relative large overlap in land-use nitrate $\delta^{15}N$ and $\delta^{18}O$ values, there was a significant land-use effect on nitrate – $\delta^{15}N$ values; nitrate from dairy streams was enriched in ^{15}N relative to other land-uses (table 4.3). No land-use relationship with nitrate – $\delta^{18}O$ values was observed (P = 0.170, SS = 33.11, *df* = 5).

Table 4.3: Mean nitrate – δ15N & δ18O values for each land-use with associated standard deviation (St. dev). The letters, a, b & c denote groupings (different at P < 0.05) based on linear mixed effects models. δ15N land-use effect was significant (P < 0.001, T = 8.89, *df* = 5) while the $\delta^{18}O$ effects was not (P = 0.170, T = 1.25, *df* = 5)

Land-use	n	mean $\delta^{15}N_{(Nitrate)}$ (‰)	$\delta^{15}N_{(Nitrate)}$ (‰) St. dev	mean $\delta^{18}O_{(Nitrate)}$ (‰)	$\delta^{18}O_{(Nitrate)}$ (‰) St. dev
Indigenous regeneration	23	4.7^{a}	1.32	3.3	3.55
Gorse and Broom	10	7.62^{b}	3.37	3.63	3
Horticulture	3	9.4^{b}	1.19	4.3	2.15
Golf course	4	7.14^{b}	2.98	4	3.19
Dry stock	16	7.28^{b}	1.97	5.28	3.29
Dairy	9	10.67^{c}	5.43	6.29	4.44

Overall across Bank Peninsula streams, there was a positive co-varying relationship between $\delta^{15}N$ and $\delta^{18}O$ values (P = <0.001, T = 7.82, *df* = 1). Within the six land-uses significant $\delta^{15}N$ – $\delta^{18}O$ covariation was only observed within all land-uses except indigenous regeneration streams (P = 0.342). The slopes of the observed $\delta^{15}N$ – $\delta^{18}O$ relationships were not significantly different (P = 0.871) (fig. 4.5). Relationships between in-stream and riparian parameters and the nitrate stable isotopic values are discussed in the following sections.

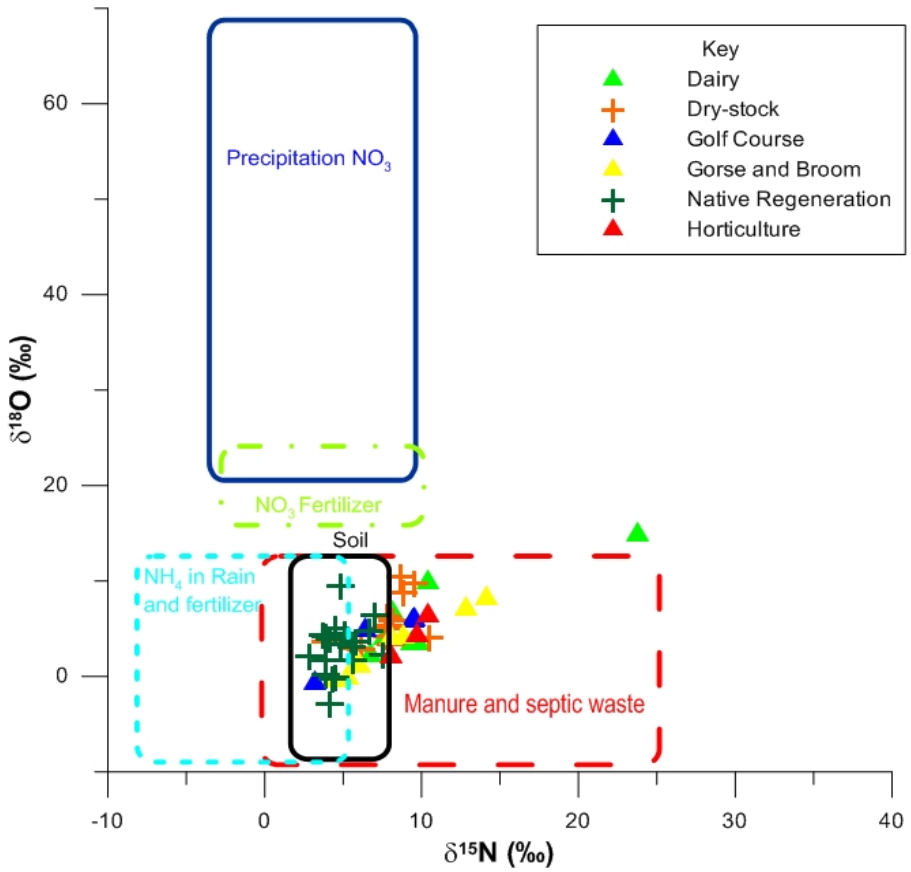

Figure 4.4: δ15N & δ18O values of nitrate from the Banks Peninsula streams. Symbols denote land-use. The boxes represent global averages of nitrate source isotopic signature ranges (Adapted from Kendall et al. 2007)

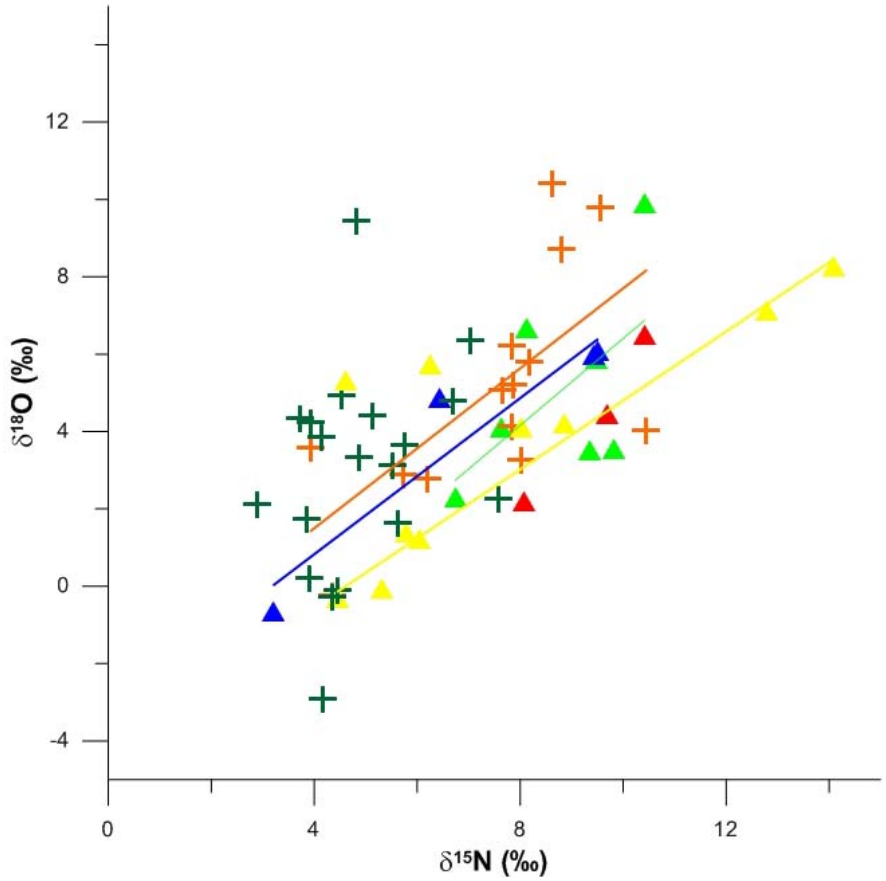

Figure 4.5: Banks Peninsula stream nitrate – δ15N & δ18O values. The figure has been magnified to emphasize intra-system land-use relationships. Lines indicate the slope of significantly covariance relationships between nitrate – δ15N & δ18O (gorse and broom: n = 8, R^2 = 0.98; golf course: n = 4, R^2 = 0.89; dairy: n = 8, R^2 = 0.81; dry stock: n = 14, R^2 = 0.43). The slopes of the four lines were not significantly different (P = 0.871). Land-uses were grouped across all three sampling seasons

4.4.5 Gorse and broom systems

Gorse and broom stream $\delta^{15}N$ & $\delta^{18}O$ values significantly covaried (r^2 = 0.579). However, when spring samples were omitted, the relationship coefficient was r^2 = 0.980. When comparing the relationships of the suite of environmental parameters with $\delta^{15}N$ & $\delta^{18}O$ values, data were analysed with

spring samples omitted as well as in its entirety (table 4.4); however, all general conclusions have been drawn from the spring omitted dataset. In terms of relationship strength (r^2) with nitrate isotope values, the top three ranked environmental parameters are all in-stream parameters (Dissolved oxygen, conductivity and phosphate concentration) (table 4.3). The highest ranked riparian parameter (fourth) was, soil C:N (r^2 = 8.07, P = 0.091), followed soil $\delta^{15}N$ (r^2 = 0.523) and longitudinal downstream distance (r^2 = 0.213). Rankings changed when the dataset was measured in its entirety; soil C:N ratio had the strongest relationship with nitrate – $\delta^{15}N$ followed by conductivity. Relationships were generally weaker for the whole (spring-included) dataset with some parameters (dissolved oxygen, nitrate concentration, pH and temperature) having alternate positive and negative relationships with nitrate – $\delta^{15}N$ and $\delta^{18}O$ values (see Table 4.4). Relationships between environmental parameters and nitrate – $\delta^{15}N$ appeared to be more affected by the inclusion of spring samples than relationships with nitrate – $\delta^{18}O$ suggesting that deviation from the relationship was more likely due to abnormally low nitrate – $\delta^{15}N$ values rather than abnormally high nitrate – $\delta^{18}O$ values (table 4.4).

Table 4.4: summary of regression analyses showing the five strongest relationships (r2) between environmental nitrate δ15N and δ18O values within gorse and broom streams. The table is separated into data from autumn and summer sampling only (i.e. spring included) and the whole dataset. + & – signs indicate the relationship between the parameter and nitrate $\delta^{15}N$ or $\delta^{18}O$. 0 indicates no relationship ($r^2 < 0.050$). Grey highlighted cells represent the relationship from which the given P-value was derived while n = analysis sample size

	Excluding Spring					Whole dataset		
	P	n	Relationship	NO_3 – $\delta^{15}N$	NO_3 – $\delta^{18}O$	Relationship	NO_3 – $\delta^{15}N$	NO_3 – $\delta^{18}O$
D.O.	**<0.001**	**8**	+/+	0.989	0.959	0/0	0.048	0.012
Conductivity	**0.005**	**8**	+/+	0.845	0.817	+/+	0.705	0.500
PO_4 Conc.	**0.008**	**8**	-/-	0.822	0.821	-/-	0.158	0.415
NO_3 Conc.	**0.061**	**8**	+/+	0.717	0.714	0/+	0.037	0.306
Soil C:N	**0.091**	**5**	+/+	0.803	0.576	-/-	0.752	0.700

4.4.6 Indigenous regeneration systems

Indigenous regeneration streams exhibited the largest range in nitrate $\delta^{18}O$ values (-2.9 – 9.5‰) and lowest mean $\delta^{15}N$ values (4.9‰ ± 1.32) of all land-use classes (fig. 4.4). While there was no correlation between nitrate – $\delta^{15}N$ and $\delta^{18}O$ values across indigenous regeneration streams, some environmental parameters did display relationships with nitrate – $\delta^{15}N$ or $\delta^{18}O$ values. Nitrate $\delta^{18}O$ values correlated positively with water $\delta^{18}O$ values (P = 0.007). The only other correlation detected was a positive relationship between nitrate – $\delta^{15}N$ and ammonium concentration (P = 0.081) (table 4.5).

Table 4.5: summary of regression analyses showing the five strongest relationships (r2) between environmental nitrate δ15N and δ18O values within indigenous regeneration streams. Grey highlighted cells represent the relationship from which the given P-value was derived and n = analysis sample size. + & – signs indicate the relationship between the parameter and nitrate $\delta^{15}N$ or $\delta^{18}O$. 0 indicates no relationship ($r^2 < 0.050$)

	P	n	Relationship	NO_3 – $\delta^{15}N$	Relationship	NO_3 – $\delta^{18}O$
Water -$\delta^{18}O$	0.006	10	0	0.026	+	0.619
NH_4 Conc.	0.081	14	-	0.200	0	0.033
NO_3 Conc.	0.149	19	0	0.045	+	0.118
Soil C:N	0.391	4	-	0.232	0	0.029
Soil $\delta^{15}N$	0.275	4	0	<0.001	0	0.024
PO_4 Conc.	0.782	19	0	<0.001	0	<0.001

4.4.7 Dairy systems

On average, nitrate from dairy streams had the highest $\delta^{15}N$ values (fig. 4.4). Dairy stream nitrate – $\delta^{15}N$ and $\delta^{18}O$ values were positively correlated (P = 0.004, r^2 = 0.8058). Four of the five strongest relationships (based on r^2 values) between environmental parameters and nitrate –$\delta^{18}O$ values were significant (table 4.6). In cases where the r^2 value for the relationships between environmental parameters and nitrate – $\delta^{15}N$ was bigger than that with $\delta^{18}O$, the relationship with $\delta^{18}O$ was still used in order to avoid the extreme leverage by the most ^{15}N enriched data point (table 4.6). The five strongest parameter relationships all were in-stream physicochemical measures (table 4.6).

Table 4.6: summary of regression analyses showing the five strongest relationships (r2) between environmental nitrate δ15N and δ18O values within dairy streams. Grey highlighted cells represent the relationship from which the given P-value was derived while n = analysis sample size. All analyses were conducted on nitrate – $\delta^{18}O$ values to account for the leverage

of the most enriched nitrate – $\delta^{15}N$ value. + & – signs indicate the relationship between the parameter and nitrate $\delta^{15}N$ or $\delta^{18}O$. 0 indicates no relationship ($r^2 < 0.050$)

	P	n	Relationship	NO_3 – $\delta^{15}N$	Relationship	$NO_3 – \delta^{18}O$
NO_2 Conc.	0.021	7	+	0.891	+	0.972
pH	<0.001	8	+	0.811	+	0.872
Temp.	<0.001	8	+	0.868	+	0.856
D.O.	0.033	6	-	0.567	-	0.716
Conductivity	0.125	8	+	0.632	+	0.364

4.4.8 Dry stock systems

Dry stock stream nitrate isotope values had the largest scatter of all six land-uses (fig. 4.5). As in dairy streams, nitrate – $\delta^{15}N$ and $\delta^{18}O$ values displayed positive covariation ($P = 0.012$, $r^2 = 0.425$). Generally the relationships between environmental parameters and nitrate – $\delta^{15}N$ and $\delta^{18}O$ values were considerably weaker than those in dairy streams (table 4.7). The longitudinal distance of the site from the stream's origin was the strongest predictor of nitrate ($\delta^{15}N$) values (table 4.7); nitrate was enriched in ^{15}N in downstream sites relative to upstream sites. Of the five strongest predictors, three were measures of riparian condition. However, only one of these was significant (canopy cover) (table 4.7).

Table 4.7: dry stock stream correlation coefficients (r2) for trends between environmental nitrate δ15N and δ18O values for the five strongest relationships. Grey highlighted cells represent the relationship from which the given P-value was derived while n indicates analysis sample size. + & – signs indicate the relationship between the parameter and nitrate $\delta^{15}N$ or $\delta^{18}O$. LDD (longitudinal downstream distance) is the meters from the stream head (origin) to sampling point

	P	n	Relationship	NO_3 – $\delta^{15}N$	Relationship	NO_3 – $\delta^{18}O$
LDD	0.003	13	+	0.567	+	0.148
PO_4 Conc.	0.043	14	+	0.012	+	0.299
Canopy Cover	0.095	14	-	0.015	+	0.215
Soil C:N	0.200	5	+	0.474	+	0.159
Soil $\delta^{15}N$	0.639	4	-	0.130	-	0.419

4.4.9 Non-agricultural anthropogenic land-use

Golf course and horticulture sites had the lowest sample sizes of the six land-uses (n = 4 & 3 respectively). While this makes hard to draw conclusions from analyses, some insight can still be gained through describing the available data. Both golf course and horticultural streams exhibited positive linear relationships between their nitrate – $\delta^{15}N$ and $\delta^{18}O$ values (r^2 = 0.895 & 0.963); however this relationship was non-significant for horticultural streams (P = 0.122), and on the significance threshold for golf course streams (P = 0.054). When the two land-use datasets were combined, the relationship between nitrate – $\delta^{15}N$ and $\delta^{18}O$ values was significant (P = 0.015). For investigating relationships between nitrate – $\delta^{15}N$ & $\delta^{18}O$ values and environmental parameters, golf course and horticulture data were combined. Of all the environmental parameters tested for relationships with nitrate isotopic values, only one had a likelihood >60% of being correct; the positive correlation between pH and nitrate – $\delta^{15}N$ (P = 0.094).

4.5 Discussion

4.5.1 Land-use effects

Nitrate-N concentrations in Banks Peninsula streams were generally lower than half of the national water quality guide lines of (10 ppm). Comparatively, nitrate concentrations in intensive agricultural streams on the adjacent Canterbury Plains ranged from 2 – 11.4 ppm (Vasquez-Menay *pers. data*), while agricultural streams on the peninsula ranged from <0.001 – 1.682 ppm. Previous research in the Cambrian Shield area, Canada has shown golf courses contribute significant levels of nitrate to stream nitrogen budgets. Nitrate-N concentrations within golf course streams ranged from 0.048 – 0.444 ppm and total N concentrations ranged from 0.427 – 1.906 ppm (Winter and Dillon 2005). These results are comparable to the observed range in Banks Peninsula golf course streams (<0.001 – 1 ppm). Similarly, nitrate-N concentrations in horticultural and indigenous regeneration streams were comparable to previously reported ranges in New Zealand. Banks Peninsula horticultural stream nitrate-N ranged between (<0.001 – 0.674 ppm compared to 0.01 – 0.9 ppm and, within indigenous regeneration streams, <0.4 ppm compared to < 0.50 ppm previously reported in the South Island New Zealand (Young et al. 2005) While no previous research has documented the relationship between gorse and broom cover and stream nitrate concentration, the presence of autumn olive (*Elaeagnus umbellate*) (an invasive legume shrub in the United States) was shown to increase stream nitrate-N concentrations from <0.001 to 0.51 ppm within-streams (Goldstein et al. 2010). However, in this study, gorse and broom streams were associated with changes in nitrate-N as large as approximately 2 ppm relative to indigenous regeneration streams. While my results show that gorse and broom land-use is a significant source of nitrate-N within-streams, the fact that reported nitrate-N ranges in all other land-uses (except of agricultural) were similar to previously reported results suggests that gorse and broom may also be a

nationally significant source. Investigating relationships within nitrate $\delta^{15}N$ & $\delta^{18}O$ values associated with these land-uses, however, allowed me to investigate relationships between land-use and nitrate more in depth.

4.5.2 Nitrate $\delta^{15}N$ & $\delta^{18}O$

Positive correlation between nitrate $\delta^{15}N$ & $\delta^{18}O$ values was observed in all land-uses except for indigenous regeneration. Positive nitrate $\delta^{15}N$ & $\delta^{18}O$ correlations are attributed to biological processes (Kendall et al. 2007). The fact that this relationship was observed across all land-uses except for regenerating indigenous forest suggests that there is some distinct difference between how nitrate is being cycled within these (relatively unperturbed) systems and all other human impacted land-uses. Further to this, the fact that the slope of this fractionation relationship is consistent across the four human impacted land-uses suggests that the cause of this fractionation is also consistent (Kendall et al. 2007; Granger et al. 2010). Biological fractionation occurs when a portion of lighter isotopes in the nitrate pool are being removed, resulting in an isotopic enrichment of the residual nitrate body. Hence, understanding what is controlling this fractionation will indicate what is controlling the removal of nitrate from streams.

Although the slope of the fractionation relationship was consistent between land-uses, the relationship fit (r^2) varied between the land-uses. Gorse and broom and golf courses had the strongest relationships (r^2 = 0.98 & 0.89 respectively), while in agricultural sites (dairy and dry stock) it was lower (r^2 = 0.81 & 0.43 respectively). Understanding fractionation relationships is highly complicated incorporating many potential variables (Xue et al. 2009; Granger et al. 2010). Nonetheless these differences in the relationship fit suggest that there are land-use differences in the controls of nitrate

processing which warrant further investigation. Below I will discuss these relationships for each specific land-use.

4.5.3 Gorse and broom systems

The significance of invasive N-fixing shrubs in terrestrial nitrogen cycles has long been recognized (Vitousek et al. 1997; Vitousek et al. 2002). However, recently research has begun to focus on the specific interactions between invasive N-fixers and adjacent water quality. In North America relationships have been shown between the cover of the invasive N-fixer Russian olive (*Elaeagnus angustifolia*) and DON export (Mineau et al. 2011) as well as riparian soil N content (Follstad Shah et al. 2010). Moreover the closely related autumn olive (*Elaeagnus umbellata*) was shown to: increase nitrate leaching within associated soil (Baer et al. 2006); elevate groundwater nitrate levels (Goldstein et al. 2010); be positively correlated with stream nitrate levels (Goldstein et al. 2009). In New Zealand, gorse has been shown to increase soil nitrate leaching within invaded plantation (*Pinus radiata*) forests in the Central North Island (Magesan 2009). Drake (2011) demonstrated that broom fixed nitrogen at a rate of 0.061 mg N per gram of dry plant biomass per day when grown in gravel beds. However, no significant nitrate leaching was detected (Drake 2011). Comparing the finding of these studies shows that invasive N-fixing shrubs impact different systems in a variety of ways. Understanding why such varied effects may have been seen will enable more thorough environmental management practices.

Two differences between the Banks Peninsula and the adjacent Canterbury Plains where Drake (2011) documented no contribution of nitrate by gorse and broom are the nutrient limiting status of the system and the soil type. The Canterbury Plains are considered P-limited while the Banks Peninsula is considered N-limited (O'Brien pers. comm.). Similarly, the effects of Russian olive detected by Mineau et al. (2011) were also in a N-limited system with characteristically low nitrate concentrations. Magesan

(2009) demonstrated a significant contribution of gorse to plantation forestry systems in the Central North Island of New Zealand where the soil is phosphorous rich due to the extensive volcanic history of the area (Rutherford et al. 1989). Goldstein et al. (2010) did not make reference to the nutrient limiting status of their system. While the other studies were in areas with established soils with organic matter, Drake (2011) stresses the point that the riverbeds used in the study were coarse gravel with negligible amounts of organic matter. The Banks Peninsula soils primarily loess soils with little organic matter (Trangmar 2003). Gorse and broom retain their fixed nitrogen within biomass (Drake 2011); however, as biomass is frequently jettisoned, over time this N-rich organic matter will breakdown potentially causing the soil N-pool to increase to the extent where inorganic nitrogen (nitrate) leaks from the system (Magesan *In press*). Both factors are likely to be contributing to the observed discrepancies but to what extent is unknown.

These uncertainties highlight the importance of understanding the causal link between invasive N-fixing shrubs and elevated stream nitrate levels. One fundamental question is whether the nitrate entering the streams is coming directly from the N-fixing shrubs or whether their presence is affecting the way in which pre-existing nitrogen within the system is being cycled. Both are plausible options seeing as these shrubs appear to be largely invading agricultural land (e.g. Baer et al. 2006; Goldstein et al. 2009; Follstad Shah et al. 2010; Goldstein et al. 2010; Mineau et al. 2011) as is the case on the Banks Peninsula. The nitrate – $\delta^{15}N$ and $\delta^{18}O$ values give insight into the mechanisms causing elevated nitrate levels in gorse and broom streams.

The fact that nitrate $\delta^{15}N$ & $\delta^{18}O$ values from summer and autumn samples fitted so tightly along the fractionation line (r^2 = 0.980) suggests that nitrate is coming from a single source. On the Banks Peninsula gorse and broom invade low-productivity agricultural land (commonly grazed by dry stock)

(Wilson 1994). If elevated nitrate in gorse and broom affected streams was a land-use legacy (potentially accentuated by the physical presence of gorse and broom) then the nitrate isotopic values would follow a similar patternn to those of dry-stock streams. If elevated nitrate levels within gorse and broom sites were a legacy effect, nitrate $\delta^{15}N$ & $\delta^{18}O$ values within gorse and broom streams would be expected to be similar to dry stock agricultural sites.

Sites sampled in spring most likely diverged from fractionation line due to low nitrate – $\delta^{15}N$ values. while this may be caused by spring sampling being conducted within a month of a significant seismic event (the September 4th magnitude 7.1 Greendale earthquake), similar anomalous relationships were not observed within other land-uses. Winter is typically the peak rainfall period within the region (Soons et al 2003). During spring soil would, thus, like higher water content and shorter residence time than summer and autumn which may impact soil biogeochemical processes (Barnes and Raymond 2010). Soil water saturation favours denitrification over nitrification (Goodale et al. 2009). No other sites, within the catchment, from which the spring samples were taken showed similar nitrate $\delta^{15}N$ & $\delta^{18}O$ values or had elevated nitrate-N levels, suggesting that unexplained soil biogeochemical processes more likely caused the anomalies. Nonetheless, the strong single source relationship observed during summer and autumn provides a unique opportunity to investigate what is controlling fractionation within gorse and broom systems.

Positive linear relationships between nitrate – $\delta^{15}N$ and $\delta^{18}O$ values have been reported widely within the literature. This relationship is attributed to either denitrification or nitrate uptake (Kendall et al. 2007). A negative relationship between nitrate concentration and nitrate – $\delta^{15}N$ is the commonly used as a rule of thumb to distinguish denitrification (Anisfeld et al. 2007; Kendall et al. 2007; Diebel and Van Der Zaden 2009; Itoh et al. 2011); whereas a positive relationships generally is attributed to

biological nitrate uptake (Kendall et al. 2007; Granger et al. 2010). Here I found that nitrate – $\delta^{15}N$ values had a strong relationship with in-stream parameters indicating that the fractionation was occurring within the streams and not the riparian soil. Further, nitrate – $\delta^{15}N$ values were positively correlated with the stream's nitrate concentration ($P = 0.0611$, $r^2 = 0.71$); suggesting that nitrate uptake was the primary removal mechanism of nitrate within gorse and broom systems. It is common, however, for multiple N-removal processes to be acting simultaneously operating (Robinson 2001; Deutsch et al. 2009); the fact that the relationship between nitrate-N concentration and $\delta^{15}N$ values within gorse and broom streams was relatively weak suggest that this is the case.

The two strongest correlates with nitrate – $\delta^{15}N$ values within gorse and broom sites were dissolved oxygen and phosphate-P concentration. These are both fundamental parameters of ecosystem metabolism (Dodds 2007) suggesting nitrate removal is reliant on stream metabolism (i.e. the production and consumption of carbon). Also, a lack of correlation between nitrate – $\delta^{15}N$ and phosphate concentration has been previously used as an indicator of the presence of denitrification (Sigman et al. 2003). These points further suggest that nitrate is primarily removed via in-stream autotrophic and or heterotrophic assimilation. Determining the relative significance of autotrophic and heterotrophic uptake was not possible here. Heterotrophic biofilm has been hypothesised to be potentially significant N sinks within lotic systems (Carpenter et al. 2008). Mineau et al.'s (2011) conceptual model hypothesising the effects of invasive N-fixer on stream N uptake suggests that the streams invaded by N-fixing shrubs are being subsidised by organic carbon in order to maintain uptake of nitrate at high concentrations. A simple framework in which the most limiting nutrient is taken up with the most efficiency is supported by the significant negative relationship between stream phosphate and nitrate – $\delta^{15}N$ within gorse streams in this study. In other words, uptake of nitrate was the most

efficient (i.e. lowest isotopic fractionation) when background phosphate levels were at their highest. Denitrification is likely to be limited to restricted areas as dissolved oxygen in Banks Peninsula streams was commonly at, or near saturation level (average across all streams was 11.1 mg/l i.e. 96.4% of saturation concentration) and the steep catchments typically do not accumulate large volumes of fine sediment.

The data show that the original isotope source values are not retained and instead are altered through biological processing within the stream and is dependent on in-stream physicochemical conditions (primarily dissolved oxygen concentration and phosphate concentration). Nitrate stable isotope values are ever increasingly being used to understand nitrate source contributions in complex mixed land-use catchments. High natural variability of nitrate isotope values has been frequently reported within studies (Xue et al. 2009). Here it is shown that over 98% of the variation in nitrate $\delta^{15}N$ and $\delta^{18}O$ values over a range of 12‰ and 10 ‰ respectively can be explained by in-stream fractionation when other physicochemical parameters are known. While inter-system differences likely negate broad applicability, correcting for biological fractionation relationships within gorse and broom systems significantly increases the accuracy of identifying nitrate source isotopic signatures. Such an approach has been applied with denitrification fractionation relationships to apportion atmospheric and sewage effluent derived nitrate (e.g. Anisfeld et al. 2007).

The fact that the soil C:N ratio correlated with nitrate – $\delta^{15}N$ (with a 90% confidence interval) suggests that riparian soil condition is affecting stream nitrate cycling (i.e. higher nitrate – $\delta^{15}N$ values associated with soils containing relatively more nitrogen). The primary path through which nitrate in entering the stream is most likely soil leaching. Soil C:N has previously been shown to be a significant predictor of nitrate leaching. The nitrate is then being fractionated once entering the stream.

4.5.4 Indigenous regeneration systems

There was no correlation between nitrate – $\delta^{15}N$ & $\delta^{18}O$ within indigenous regeneration streams which also displayed the largest over all scatter within their isotopic values . The strongest relationship detected with nitrate isotopic values across indigenous regeneration streams was the positively correlation of nitrate – $\delta^{18}O$ with water – $\delta^{18}O$ values ($r^2 = 0.619$). As a rule of thumb, during nitrification nitrate receives one O atom from the atmosphere [$\delta^{18}O_{Air} = 23.5‰$] and two from the associated water body (Kool et al. 2007). Thus the relationship between nitrate – $\delta^{18}O$ and water – $\delta^{18}O$ seen here in indigenous regeneration streams suggests that nitrification is happening at a local scale with little, biological fractionation occurring after this. Less nitrate isotopic fractionation occurs at low nitrate concentrations due to more efficient uptake (Earl et al. 2006). Biological fractionation effects are nullified under two conditions: when there is no biological activity on the given atomic mass, or when biological demand for the given atomic population is so great that the entire atomic mass will be assimilated. As nitrate is the predominant form of inorganic nitrogen within freshwater systems and nitrogen is an essential element for sustaining all known life forms (Galloway 2004), it is highly unlikely that there is no biological demand for nitrate within indigenous regeneration streams given the characteristically low concentrations and assumed N-limited status of the region. The tight relationship between nutrient cycling, nitrification and uptake is further supported by the fact that the strongest correlate with nitrate – $\delta^{15}N$ was ammonium concentration ($r^2 = 0.200$). Localised nitrification with rapid uptake and a discernible relationship with ammonium indicates that nitrogen is cycled tightly (between organic and inorganic phases) within indigenous regeneration streams. Extensive research indicates that nitrate uptake is highest when background concentrations are low (Peterson et al. 2001; Valett et al. 2008). Low discharge forested streams characteristically have rapid uptake rates, indicative of tight nitrogen cycling (Peterson et al. 2001). While the conclusions drawn here fit well with the wider

literature body, it also indicates that indigenous forested systems with tight nitrogen cycling (at least within this study area) are likely to display a large scatter in their nitrate – $\delta^{15}N$ & $\delta^{18}O$ isotopic values making it hard to determine a indigenous regeneration land-use nitrate – $\delta^{15}N$ & $\delta^{18}O$ isotopic signature.

While a significant portion of the variation in nitrate $\delta^{18}O$ isotopic values appears to be associated with H_2O - $\delta^{18}O$ isotopic values (62%), variation in the $\delta^{15}N$ values is less clear cut. While ammonium-N concentration may be in part controlling nitrate $\delta^{15}N$ values, a large degree of scatter was still observed. This may be due to either the stochastic nature of nitrate sources, or a historical land-use legacy affect. When nitrate concentrations are low multiple smaller (localised) nitrate sources will not have their isotopic signature dampened out by one overwhelming source (Robinson 2001). it is likely that most systems do receive various stochastic sources of nitrate albeit in small quantities (Loreau and Holt 2004; Galloway et al. 2003) For example it has been shown that fish distribution can significantly influence nutrient cycling and localised concentrations through feeding in one habitat then resting (excreting) in another (McIntyre et al. 2008; Small et al. 2011). Without dilution from a significant anthropogenic source, the nitrate – $\delta^{15}N$ values of these sources are more likely to be detected in (Robinson 2001; Kendall et al. 2007).

Conversely, the background variation could be attributed to historic land-uses. Land-use legacies have been shown to have strong effects on stream communities in western North Carolina (USA) (Harding et al. 1998). Historic seabird colonies have been shown to leave biogeochemical imprints on the soil of Pacific Islands after they have been removed by invasion of predatory mammals; however, over 100 year time scales historic N was seen to have been lost from the system due to its solubility (Wardle et al. 2009). While evidence suggests the Banks peninsula historically (> 150 years before present) supported significant seabird breeding colonies, evidence suggests that they were not as

concentrated as those reported by Wardle et al. (2009) (Hawke 2003). During the 20th century, the predominant land-use on the Banks Peninsula was dry-stock agriculture (Harding 2003). Dry-stock nitrate – $\delta^{15}N$ values were significantly higher those within indigenous regeneration sites (nitrate – $\delta^{15}N$ = 7.28 ± 1.97 ‰ (dry stock) compared to nitrate – $\delta^{15}N$ = 4.70 ± 1.32 ‰ (indigenous)). It is difficult to draw robust conclusions on the cause of the observed scatter in nitrate $\delta^{15}N$ isotope values; however, from the evidence available, I suggest that the presence of multiple localised sources of reactive nitrogen (as discussed above) is the primary cause of this variation.

4.5.5 Agricultural systems

While dairy and dry stock streams both displayed significant positive nitrate – $\delta^{15}N$ & $\delta^{18}O$ fractionation relationships, they differed in terms of the strength of that relationship (r^2 = 0.81 & r^2 = 0.43 respectively), average nitrate concentration (nitrate-N = 0.48 ± 0.48 ppm and nitrate-N = 0.26 ± 0.34 ppm respectively) and their average nitrate – $\delta^{15}N$ values (nitrate – $\delta^{15}N$ = 10.67 ± 5.43‰ compared to nitrate – $\delta^{15}N$ = 7.28 ± 1.97‰ respectively). Moreover, within dairy sites the five strongest correlations between nitrate – $\delta^{15}N$ & $\delta^{18}O$ values and environmental parameters were all in-stream parameters (nitrite-N concentration, pH, dissolved oxygen and conductivity) whereas in dry stock systems three of the five strongest predictors were riparian (canopy cover, soil-C:N and soil- $\delta^{15}N$). This suggests that the systems differ in the amount of reactive nitrogen they receive and the way in which the system is processing it. Anecdotally these two land-uses differ in their fertilizer application rates, however, the biggest contrast between them is their stocking rates, 25.25 stock units per Ha and 7 units per Ha in dairy and dry stock areas respectively. This suggests that diffuse stock effluent runoff is the primary difference between the two systems. This is supported by the fact that nitrate – $\delta^{18}O$ values fell outside of the range of reported nitrate fertilizer $\delta^{18}O$ values (Kendall et al. 2007) and that nitrite and

ammonium concentrations were higher in dairy sites than all others. Nitrite is an intermedairy compound during nitrification (Galloway 2003); that it was in excess and correlated positively with nitrate – $\delta^{15}N$ values suggests that nitrification of ammonium (from stock manure) is the primary control on biological uptake in dairy streams. Further to this, because the oxidation of ammonium releases H^+ ions, pH is commonly affected by nitrification (Galloway et al. 2004); pH correlated positively with nitrate – $\delta^{15}N$ values in dairy streams. A dairy cow excretes on average 0.51 kg N per day (Collins et al. 2007); seeing as none of these sites have riparian fencing, it seems logical that a significant portion of this may enter the stream via direct surface runoff. Animals are efficient nitrogen processors which can move reactive nitrogen in an ecosystem more rapidly and in opposing directions to that which gravity can (Robinson 2001; Galloway et al. 2003; Loreau and Holt 2004). Previous stable isotopic studies of stream nitrate have given evidence to support this (Kendall et al. 2007; Xue et al. 2009). Riparian fencing would likely reduce the primary source of nitrate entering dairy streams.

Conversely, within dry stock systems nitrate appeared mainly to be entering the stream via soil leaching. Soils with low C:N ratios (i.e. relatively more nitrogen) are more susceptible to leaching (Stevenson et al. 2010); soil-C:N correlated positively with nitrate – $\delta^{15}N$ values in dry stock streams as did soil –$\delta^{15}N$ values. This suggests that observed nitrate fractionation within dry stock streams is occurring within the soil prior to entering the stream. While stock excretion is still likely to the primary source of nitrate within these sites (Collins et al. 2007; Parfitt et al. 2008), due to the lower stock densities, it appears that is being processed in the soils prior to entering the stream. As indicated by the differences in the responses of dairy and dry stock systems, these different nitrate source pathways may be significantly affecting the stream's response (Galloway et al. 2003; Dodds 2007).

4.5.6 Non-agricultural anthropogenic land-use systems

Nitrate stable isotopic data was only able to be attained from three horticultural samples and four golf course samples. While this meant that rigorous statistical analysis of nitrate isotopic values eluded these land-uses, some inferences could still be drawn about N-cycling in these systems. Nitrate concentrations within golf course sites in this study (nitrate-N = 0.33 ± 0.32 ppm) were similar to findings from previous research which has shown a link between nitrate leaching and fertilizer application (Winter and Dillon 2006). While previous research indicates that horticultural land can leach significant levels of nitrate as well (Stevenson et al. 2010), nitrate-N concentrations within horticultural sites within this study were lower than all other anthropogenic land-uses. While nitrate – $\delta^{15}N$ & $\delta^{18}O$ values in both golf course and horticultural land-uses appeared to follow positive linear fractionation relationships, the small sample size (n = 3) for horticultural streams cautions me against making any inferences as to the isotopic relationships within these systems. Within golf course streams, a relatively strong nitrate – $\delta^{15}N$ & $\delta^{18}O$ positive correlation relationship (r^2 = 0.89, n = 4) was observed, indicating a potential dominant source of nitrate within these systems. When the horticultural and golf course datasets were combined no significant relationships were observed. This may indicate that although superficially these systems are subjected to similar nitrate sources they are in fact quite responding to nitrate sources. When investigating golf course nitrate – $\delta^{15}N$ values independently, none of the environmental parameters significantly correlated with either nitrate – $\delta^{15}N$ or $\delta^{18}O$ values; however, the strongest relationship observed was between nitrate – $\delta^{15}N$ and pH. Nitrate – $\delta^{15}N$ & $\delta^{18}O$ values within golf course sites was within the reported range of ammonium fertilizers (Kendall et al. 2007), which is the predominant form of reactive N applied to golf courses in this study. Ammonium components within the fertiliser are water soluble and readily nitrified in freshwater systems (Robinson 2001; Dodds 2007). However more samples are required to draw conclusions about the relationship.

4.6 Conclusion

Controlling nitrate levels within catchments is a significant issue facing environmental managers in the 21st century. This in confounded by the sometimes ambiguous relationship between land-use and nitrate levels and the fact that not all nitrate sources within the catchment may have been identified. This study exemplifies this issue by demonstrating that the land-use which was associated with the highest nitrate concentrations (fallow land invaded by gorse and broom) was previously not recognized as a nitrate source within the study area. Although other international studies have shown relationships between invasive N-fixing shrubs and elevated nitrate stream levels, this was the first to use nitrate – $\delta^{15}N$ & $\delta^{18}O$ stable isotopic analyses to indicate that this excess nitrate was in fact being fixed by the plants themselves and not a land-use legacy effect. Gorse and broom are aggressive invaders of agricultural land and riparian margins within the Banks Peninsula as well as nationally and internationally, thus should be considered within future water quality management projects. While only gorse and broom streams had significantly higher nitrate concentrations than indigenous regenerating forest sites, all anthropogenic land-uses displayed positive linear nitrate – $\delta^{15}N$ & $\delta^{18}O$ relationships. At least within gorse and broom systems, this biological fractionation appears to be primarily controlled by nitrate uptake. However the physicochemical controls on this biological fractionation varied substantially between land-uses, suggesting that there were land-use specific differences in the controls of nitrate removal. The findings shown here should be of considerable interest to both environmental managers and scientists both within New Zealand and internationally. Beyond the implications of N-fixing shrub derived nitrate, through providing nitrate – $\delta^{15}N$ & $\delta^{18}O$ data from a southern hemisphere system this study will also greatly enhance the wider general applicability of future nitrate stable isotopic meta-analyses and isoscapes projects.

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Chapter 5: Using stable isotopes to identify lotic invertebrate responses to land-uses of contrasting nitrate inputs

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5.1 Abstract

Land-use changes are among the most significant threats to the healthy functioning of lotic ecosystems. Land-use change is associated with various stressors of which nitrogen runoff is potentially the most pressing. However, measuring the sources and mechanisms of these impacts can be highly problematic; due, in part, to streams commonly being subjected to multiple interacting land-use stressors and widely varying responses of differing trophic pathways within lotic systems. $\delta^{15}N$ & $\delta^{13}C$ stable isotopic analysis of food resources and biota within the lotic system provide a powerful way to investigate various community responses to multiple land-use stressors using a single comparable currency. Further, recent advancements in stable isotope analyses have enabled researchers to elucidate consumer diet composition (isotope mixing models) and community wide trophic responses (isotope niche indices). In this study I used these tools to investigate invertebrate community responses to four land-uses: dairy agriculture (high intensity), dry stock agriculture (low intensity), gorse and broom (invasive N-fixing shrubs) and indigenous regenerating forest (reference conditions) across 35

sites on the Banks Peninsula, South Island New Zealand. Four primary consumer functional feeding groups (collector browsers, filter feeders, grazers and facilitative shredders) and secondary consumers (predators) were present across all land-uses. Lotic invertebrate responses to land-use included a reduced dietary intake of coarse particulate organic matter (CPOM) across all sites relative to regenerating indigenous forest sites and a larger community trophic niche (range in $\delta^{13}C$ values) in dairy and gorse sites relative to native regeneration and dry-stock sites. Land-use change lead to invertebrates having less CPOM available and subsequently feed on a wider range on trophic channels. However, no clear relationships were observed with these invertebrate trophic responses and individual land-use stressors (i.e. nitrate concentration or light availability), suggesting that trophic responses resulted from complex interactions between these stressors much in the same way these factors interacted to affect nitrate removal. Nonetheless, average $\delta^{15}N$ values for the whole invertebrate communities were lower in regenerating indigenous forested sites than all other sites, indicating that land-use induced changes to in-stream nitrogen cycling leaves a $\delta^{15}N$ imprint on the invertebrate community.

Despite the realized complexity of lotic community responses to land-use, this study demonstrates specific changes in the how invertebrates are utilizing basal resources across land-uses. It also shows that invertebrate $\delta^{15}N$ values may be powerful long term indicators of land-use impacts on nitrogen cycling within streams. Using stable isotopic parameters to quantify lotic system land-use impacts presents a potentially powerful tool for freshwater management.

5.2 Introduction

Land-use change is one of the primary drivers of the global decline in natural ecosystems and the services they provide (Vitousek et al. 1997; Sala 2000; MEA 2005). Nowhere else is this more pronounced than in freshwater ecosystems (Carpenter 1992; Allen 2004; Woodward 2009).

Furthermore, freshwater ecosystems, are biodiversity hotspots (Dudgeon et al. 2006) and provide ecosystem services which are of vital importance to society (Karr 1999). Lotic systems (running water) are particularly vulnerable to land-use impacts due to their inherent close proximity to and dependence on the terrestrial catchment (Allen 2004). Over the last century, New Zealand has been subjected to extensive deforestation and conversion to pastoral land (Hall et al. 2001; Harding 2003). This patternn has been observed around the world and usually results in significant impacts on associated lotic ecosystems (Allen 2004). Land-use impacts on streams can manifest in a variety of ways such as; reduced organic matter input, increased sedimentation, light and heat stress, and toxins and increased nutrient runoff (Carpenter 1998; Allen 2004; Matthaei et al. 2006). While all of these disturbances cause significant deleterious effects, increasing nutrient additions (specifically nitrate) is acutely important to freshwater systems. Nitrate pollution has been linked to; loss in diversity of stream communities (Frost et al. 2005; Hall et al. 2001; Singer and Battin 2007), changes in feeding interactions between consumers and resources within the community (Davis et al. 2010; Grover 2004; Frost et al. 2005); alteration of the balance of respiration and primary production (Dodds 2007; Ventura et al. 2008); impacts ecosystem functions (Antón et al. 2011; Benstead et al. 2009; Bernot and Dodds 2005; Grover 2004; Ventura et al. 2008). However, nitrate stresses invariably co-occur with other land-use stressors. Thus, quantifying its effects and interactions with other stressors is complex and problematic (Karr 1999; Weijters et al. 2009; Woodward 2009). As a result of these complex interactions invertebrate community based measures are commonly used as integrated long term indicators stream response to land-use (Quinn and Cooper 1997; Karr 1999; Sandin and Solimini 2009).

Land-use stressors result in reductions in biodiversity, loss of sensitive taxa, changes in relative abundances and altered trophic interactions within invertebrate communities in impacted streams (Hall

et al. 2001; Battin and Singer 2007; Sandin and Solimimi 2009; Weijters et al. 2009). Traditionally studies have focused on structural community responses (such as community composition and species richness) (Meyer 1997); however there is an increasing focus on functional responses of streams (Sandin and Solimini 2009). Functional responses refer to the processes performed by species within the community such as nitrogen cycling or processing of organic matter (Jax 2005). Functional responses provide a mechanistic understanding system response to land-use but are often difficult to quantify. Assigning organisms to functional groups is a way of indicating functional responses from structural measures. Subsequently, classing invertebrates by functional feeding groups has been a commonly used in studies investigating lotic land-use impacts. The simplicity of functional feeding group classifications for quantifying complex trophic interactions occurring with the stream has resulted in them being widely popular within the literature (Meyer 1997; Sandin and Solimini 2009). However, the use of such surrogate measures do not always confer the actual links between structure and function and are often based on too tenuous assumptions (Winterbourn 2004; Sandin and Solimini 2009). Robust indicators which can couple structure and functional responses will significantly benefit management of land-use effects and allow for a more holistic management approach (Clapcot et al. 2010).

$\delta^{15}N$ & $\delta^{13}C$ stable isotope analyses of food resources and organisms across functional feeding groups within a lotic community holds significant potential for integrating structural and functional responses. Stable isotopic analyses are an integrated approach which traces carbon and nitrogen molecules as they are transferred through the various components of a foodweb (Sharp 2007). Invertebrates will subsequently have $\delta^{15}N$ & $\delta^{13}C$ signatures which reflect their diet (Martínez del Rio et al. 2009), providing a means for comparing changes in lotic community diet across land-uses. The two general assumptions within stable isotopic foodweb ecology are that trophic transfers (i.e. one organism

consuming another) results in an isotopic enrichment of $\delta^{15}N \approx 3.4$ ‰ and $\delta^{13}C \approx 1$ ‰ (Vanderklift and Ponsard 2003) and differing photosynthetic pathways result in characteristically distinct $\delta^{13}C$ signatures (Marshal et al. 2007). When plotted out in isotopic space, $\delta^{13}C$ signatures will indicate trophic source while $\delta^{15}N$ signatures are a reference for trophic level. Numerous studies have successfully applied stable isotopic analyses, primarily identifying basal resources contributions and investigating trophic transfers within foodwebs (Winterbourn 2004; Finlay and Kendall 2007). Advancements in the analysis of isotopic data has meant that trophic source partitioning between multiple potential sources and community wide measures of trophic diversity are now possible (Layman et al. 2007; Parnell et al. 2010; Jackson et al. 2011). These essentially allow the flow of carbon and nitrogen through foodwebs to be both visualized and quantified.

Furthermore, several studies have shown that invertebrate $\delta^{15}N$ values can be useful indicators of nutrient cycling changes associated with land-use (Diebel and Vander Zaden 2007; Clapcott et a. 2010; Clapcott et al. 2011). Understanding links between the processing of nitrate within an ecosystem and trophic exchanges within the foodweb is a well recognized research objective (Galloway et al. 2003; Marcarelli et al. 2011).

This study used stable isotopic analyses of invertebrate community functional feeding groups to investigate: i) How the dietary importance of various basal trophic resources to invertebrate communities are impacted by land-use ii) The factors driving these basal resource dietary changes or lack thereof iii) Whether land-use impacts are reflected in the stable isotope signatures of the invertebrate communities? These hypotheses were tested across four land-uses; indigenous regenerating forest (reference conditions), dry-stock agriculture, dairy agriculture and fallow land invaded by exotic N-fixing shrubs gorse (*Ulex europaeus*) and broom (*Cytisus scoparius*). Further to this,

invertebrate stable isotope data only was collected from horticulture and golf course streams to further compare land-use patternns. Agricultural and horticultural land-uses have previously been shown to be significant sources of nitrate to lotic systems (Galloway et al. 2004) as have golf courses (Winter and Dillon 2005) and invasive N-fixing legumes (Goldstein et al. 2005). This was investigated in-streams across six catchments on the Banks Peninsula, South Island New Zealand where it has been shown that gorse and broom and dairy land-uses have the highest stream nitrate-N concentrations respectively (Stewart unpublished data). This essentially provides a gradient of low to high nitrate concentration over both open and closed canopy streams.

5.3 Methods

5.3.1 Study area

Banks Peninsula is the eroded remains of a late Tertiary volcanic complex on the east coast of New Zealand's South Island (fig. 5.1). Initially it was an island; however through the progradation of the Canterbury Plains, eastwards from the Southern Alps, it has been intermittently attached to the mainland since the late Quaternary (Soons et al. 2002). During glacial periods wind-deposited loess derived from the Southern Alps have accumulated on the slopes of the eroded basaltic volcanoes (Wilson 1994; Eikaas et al. 2005). Average elevation is 300m – 700m ASL (with Mt Herbert forming the highest point at 920 m ASL), while rainfall ranges from between 600 mm per year to approximately 2000 mm per year at the heads of the south-eastern valleys (Soons et al. 2002). Streams typically radiate out from the summits towards the sea and are generally third order at the river's mouth. Catchments are characteristically steep sided with coarse boulder substrate. There are over 100 distinct catchments within the 1102 km^2 region (Harding 2003).

Prior to human arrival, approximately 1000 years ago, the eastern portion of Banks Peninsula was covered largely in podocarp forest with isolated pockets of beech forests occupying some of the drier eastern and harsher high elevation areas (Soons et al. 2002; Harding 2003). However, anthropogenic deforestation, particularly since the arrival of European settlers in the mid-1800's, has drastically reduced the forest cover through both logging and burning. By 1900 less than 0.001% of the estimated 1840 forest cover remained (Harding 2003). Indigenous forest is now largely restricted to small stream valleys; from here is regenerating outwards (Wilson 1994; Harding 2003). Today, the primary land-use is low intensity dry stock agriculture (e.g., sheep). However, there are also significant areas of dairying and horticulture. Gorse and broom vegetation invaded large areas and there are several small towns and golf courses. While the area has a rich tradition of dairy farming, much of this was converted to low intensity dry-stock farming over the last half century (Ogilvie 2007).

5.3.2 Land-use classifications

Land-use classifications were initially based on GIS land cover and agricultural practice databases (*Ministry for the Environment database 2009*). Selected sites were then ground-truthed through field reconnaissance. Distribution of each land-use within the six study catchments is displayed in figure 5.1

Indigenous regeneration

Indigenous vegetation regenerations sites were considered areas of secondary indigenous growth or more mature (Podocarpus species > 100 years old). The dominant secondary growth vegetation is Kanaka (*Kunzea ericoides*) as well as occasional broadleaf (*Griselinia latoralis*) and *Podocarpus* species. This is commonly associated with an understory of largely *Coprosma* and *Dracophyllum* species, five finger *(Pseudopanax aboreus*) as well as a variety of other sub-canopy species common to New Zealand

Indigenous forest. A variety of fern species commonly form the ground cover (Wilson 1994; *pers. obers.*).

Gorse (Ulex europaeus) and broom (Cytisus scoparius)

Gorse and broom are functionally interchangeable in terms of their growth, shade tolerance and N-fixing (Drake 2011; Magesan *in press*); thus were considered a single classification for this study as is consistent with land cover information. Areas of gorse and broom were defined by the Land Cover Data Base (Ministry for the Environment 2009) as those in which they were the dominant vegetation cover (i.e. >60%). When areas are invaded by gorse and broom, it will form a dense canopy forming a monoculture with a humus groundcover (Wilson 1994; Magesan *in press*).

Horticulture

Horticultural land consisted of managed perennial commercial trees. Of the two sites sampled, one was a stone fruit orchard comprised primarily of peach, apricot and cherry trees. The other site was a nursery which specialised in *Rhododendron* cultivars with a small standing of New Zealand indigenous shrubs as well.

Golf course

Two golf courses were also included as these might represent areas of high human fertiliser additions which could influence nearby streams.. The golf courses consisted of extensive areas of intensively managed exotic grassland with small areas of (primarily exotic) trees. Both courses had similar greens management practices (Cotton pers. comm.; Miller pers. comm.). The primary fertiliser applied in these systems was Country Club 18-1.3-15 by Lebanon products. It contained ammonium-nitrate (7.4%), water soluble methylene urea (6.8%) and organic nitrogen compounds (6.5%).

Dry stock agriculture

Dry stock agriculture is used to describe stock which are not milked. Primarily this consists of beef cattle and sheep. Dry stock agriculture is generally considered low intensity land management because of the characteristically low stocking rates. The average stocking rate for the Banks Peninsula is 7.0 stock-units per ha (Ogilvie 2007).

Dairy agriculture

Dairy farming in New Zealand is primarily on intensively managed exotic grassland. Socking rates are increasing with approximately3.2 dairy cattle per hectare (25.5 Stock-units/ha) (Clark et al. 2009). Herds are primarily made up from Jersey and Frisian breeds and milked at a centralised milking shed twice daily.

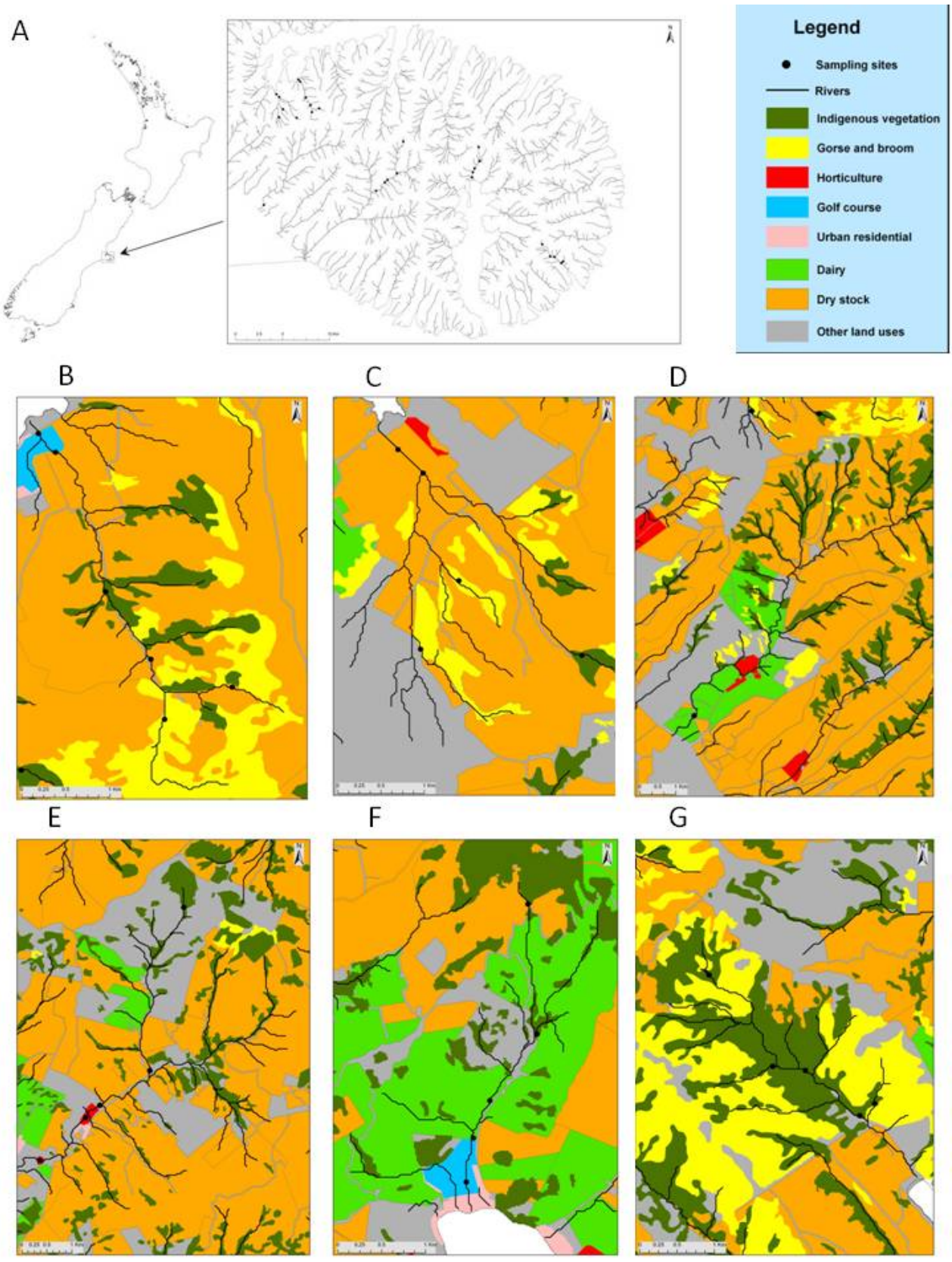
A
Legend
Sampling sites
Rivers
Indigenous vegetation
Gorse and broom
Horticulture
Golf course
Urban residential
Dairy
Dry stock
Other land uses
B
C
D
E
F
G

Figure 5.1: Study sites on the Banks Peninsula and land-use within the study catchments. All study sites within the six catchments are shown. The catchments are: B) Te Wharau (n = 6), C) Waiake (n = 5), D) Kaituna (n = 1), E) Hukaika (n = 5), E) Pawsons (n = 4), F) Narbey (n = 5). Grey areas represent a mosaic of land-uses which were not investigated in this study; primarily this consisted of leased out grazing land unspecified lifestyle properties and production forestry.

5.3.3 Data collection

A total of 35 sites were sampled across six land uses in six catchments (Table 5.1). At each site a 20m reach was sampled. Sampling was conducted over 2010 during summer and spring. Organic ecosystem components (invertebrates and basal resources) were collected for stable isotopic analyses ($\delta^{13}C$ & $\delta^{15}N$). Sites were characterised by their physico-chemical parameters (dissolved oxygen and pH), concentration of the primary growth limiting ions (nitrate, nitrite, ammonium and phosphate) and degree of canopy cover. Physico-chemical parameters were measured in-situ using a Cyberscan DO300 (Eutec Instruments) and a Cyberscan PC300 (Eutec Instruments). Ion concentrations were measure from grab-water samples and measured at the University of Canterbury for concentrations of Nitrate (NO_3^--N), nitrite (NO_2-N), Ammonium (NH_4^+-N) and phosphate (PO_4-P) using the *EasyChem* Automatic sequential Colorimetric analyzer (SYSTEA) following the Lachat method. Canopy cover was taken from visual estimates following Harding et al. (2009). A summary of these parameters within each land-use is shown in table 1.

Table 5.1: stream characterisation compared between land-uses. Ion concentrations are land-use mean values reported in elemental parts per billion (ppb) and dissolved oxygen in mg/L. Highlighted cells indicate the three highest mean nitrate concentrations. Streams classed as high canopy cover had >80% channel shading; low canopy cover streams had <30% channel shading.

Land-use	n	Canopy cover	NO_3 conc.	PO_4 conc.	NO_2 conc.	NH_4 conc.	Dissolved oxygen	pH
Indigenous regeneration	12	High	170.50	13.13	16.09	20.08	11.44	6.86
Horticulture	2	Low	201.82	20.26	1.90	11.91	11.44	7.08
Dry-stock agriculture	10	Low	263.48	14.20	3.62	24.34	10.57	7.04
Golf course	2	Low	333.54	34.60	3.42	20.09	11.41	6.68
Dairy agriculture	4	Low	480.35	108.68	15.00	124.58	12.25	6.73
Gorse and broom	5	High	1021.41	12.96	7.28	26.54	9.93	7.37

Ecosystem components were collected for $\delta^{13}C$ & $\delta^{15}N$ analysis. In order to measure changes in trophic resources across land-uses, biofilm, FPOM (fine particulate organic matter) and CPOM (coarse particulate organic matter) were collected from each site. Biofilm is the best representation of in-stream production while CPOM is detritus which has entered the stream from the riparian environment. Biofilm (composite bacteria fungi and algae) was collected by scraping 3 or more cobbles (>50 mm) which were haphazardly selected. Each scrapings were collected on 45 µm GFF filter paper. Photosynthetic plant parts (i.e. leaves, blades and green stems) of riparian vegetation (both emergent and true riparian) were collected from plants within 5 m of the stream bank. Vegetation sampling was contained to within 5m of the stream bank as it has been previously shown that plants within this area contribute the vast majority of input to a stream's allochthonous matter budget (Deegan and Ganf 2008). All sample material were

stored on ice in a dark container for up to 10 hours and then transferred to a freezer (-20°C) until analyses. FPOM was collected on the 45 µm GFF filters which were used for the filtered water samples. Once sufficient water sample had been collected, further water was flushed through the filter until the filter was visually stained. Filters were stored chilled and in the dark until further analysis in the laboratory. CPOM was collected from the kick net sample (500µm mesh). This meant the sample represented a composite of matter from various micro-habitats within each site. Invertebrates were collected using a combined kick net sample. Kick net samples were taken by disturbing the stream benthos in a variety of the micro-habitats within the stream at the specific site (i.e. channel thelweg, riffle, run, pool, stream bank). Invertebrates were picked in the field to ensure that enough of the ubiquitous taxa were present for subsequent stable isotope analysis. Predatory invertebrates were stored in separate vials to prevent post-collection predation. Invertebrates were stored in water in a polyethanol container on ice in dark conditions until freezing.

5.3.4 Laboratory processing

In the laboratory, invertebrates were sorted to genus level using the Winterbourn et al. (2006) guide. Identified invertebrates were assigned a functional feeding group based on predetermined gut analyses and morphological traits (Harding pers. comm.). Invertebrates were divided into five Functional feeding groups; collector-browsers, filter-feeders, shredders, grazers and predators. The caddisfly *Olinga* are known to contain two species of which the larvae are not possible to distinguish between yet each species can fill distinct functional roles (i.e. grazing biofilm, collecting particulate organic matter of shredding leaf detritus) (Burrell and Ledger 2003). Thus, this genus was considered facilitative shredders.

In the laboratory, invertebrates' guts were removed in order to remove any bias associated with variation in non-metabolized gut content. Material was then oven drying at 50°C for >48 hours. After drying, all samples were homogenized into a fine. Liquid nitrogen was added in order to aid grinding. Soil samples were dried in the oven after which any root material present was removed. Samples were then homogenized and passed through a sieve to exclude coarse (>0.1mm) inorganic sediment.

Powdered organic samples were then prepared for dual $\delta^{13}C$ & $\delta^{15}N$ stable isotopic analysis. As filter samples generally have a relatively high ratio inorganic associated material (the glass fibre filter material), it is imperative to have as high a concentration of sample to filter material as possible. Hence, sample free areas of filter paper were removed and only the remnant sample-rich filter was encapsulated for analysis. Subsequently, N content could only be calculated as a ratio relative to C. All organic samples were analyzed for $\delta^{13}C$ & $\delta^{15}N$ and C:N ratio using an elemental analyzer attached to a Delta V plus Thermo-Finnegan isotope ratio mass spectrometer at the University of Canterbury Stable Isotope Laboratory. All analyses were compared against international standards Pee Dee Bee (PDB) for carbon and Air for nitrogen. All measurements were expressed in the standard delta (δ), per-mil (‰) relative to the international standard. Analytical precision for $\delta^{15}N$ was approximately 0.05‰ and 0.01‰ for $\delta^{13}C$.

5.3.5 Data analysis

Data were analysed in R version 2.13.0 (2010). The relative contribution of the three primary basal resources (CPOM, FPOM and biofilm) to invertebrate communities was calculated at each site using the SIAR package isotope mixing model within R (Parnell et al. 2010). Trophic enrichment factors were assumed to be $\Delta^{15}N$ = 3.4‰ and $\Delta^{13}C$ = 1‰ (Vanderklift and Ponsard 2003; Parnell et al. 2010). Invertebrates within the communities were divided into functional feeding groups for mixing model

analysis; diet proportions for each resource were taken as the mean value of all individuals with that functional feeding group.

Invertebrate community isotopic niches were calculated for each site. Isotopic niche indices provide an integrated analysis which can act as proxies to compare the range of trophic channels contributing to a foodweb as well as the number of trophic transfers within it (Layman et al. 2007).The only isotopic niche index used in this study f the range of $\delta^{13}C$ (C-range). This is because the dataset consisted of only approximately two trophic levels; seeing as N-range and convex hull area variation in these parameters would be superfluous and thus they were deemed unnecessary (Layman et al. 2007).

Due to the non-orthogonal experimental design, mixed effects models were used for all analyses. Mixed effects models provide powerful analysis for testing interactions between predictors within non-orthogonal data (Crawley 2007). Overall gross differences in invertebrate community $\delta^{13}C$ and $\delta^{15}N$ values were compared across the six land-uses with stream characteristics (Table 1) included in the models. Resource dietary contributions and niche indices were compared in the same fashion. All reported P values are pseudo-p scores based model comparison; models were compared with a term present and omitted using maximum likelihood. The probability of difference between the two models (P value) represented the significance of that term (Crawley 2007).

5.4 Results

5.4.1 Stoichiometry of basal resources

Three basal food resources (biofilm, FPOM and CPOM) were measured. C:N ratios of all resources were lower in gorse and broom and dairy streams than indigenous regeneration and dry stock streams (fig. 5.3). The C:N ratios of biofilm and FPOM were unaffected by land-use or water nitrate

concentration (Table 5.2). CPOM had significantly more C relative to N than FPOM and biofilm ($p < 0.001$). Biofilm had the lowest C:N ratio (i.e. highest relative N content); however this was not significantly different from FPOM (fig. 5.2). CPOM from dairy streams had a lower C:N ratio than that from other land-uses, however this effect was not quite significant ($P = 0.054$) (Table 5.2). Within gorse and broom streams only, the C:N ratio of CPOM decreased with increasing stream nitrate concentration (Table 5.2).

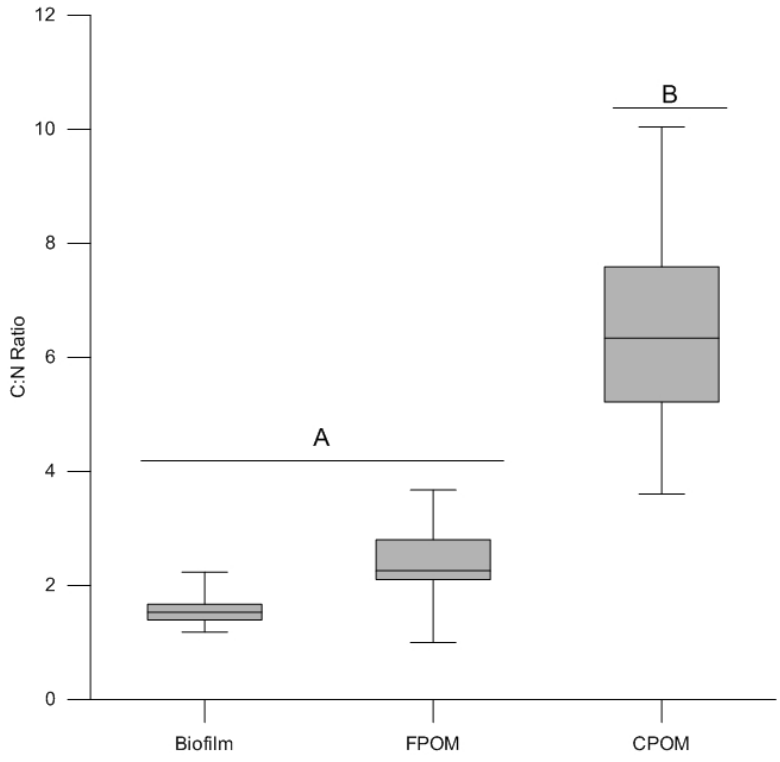

Figure 5.2: Mean C:N ratio of the primary resources. CPOM had significantly higher proportion of C relative to N than biofilm and FPOM ($p < 0.001$, $F = 7.23$, $n = 31$ Whiskers represent upper and lower quartiles

Table 5.2: summary of the linear mixed effects model showing the relationships of the three basal resources' C:N ratios with land-use, nitrate-N concentration and the interaction between them

	df	*T value*	*P*
Biofilm			
Land-use	4	0.56	0.58
NO_3-N	1	0.76	0.455
Land-use x NO_3-N	4	0.87	0.392
FPOM			
Land-use	4	1.6	0.126
NO_3-N	1	1.1	0.284
Land-use x NO_3-N	4	1.12	0.228
CPOM			
Land-use	4	3.08	0.054
NO_3-N	1	1.73	0.101
Land-use x NO_3-N	4	2.11	0.049

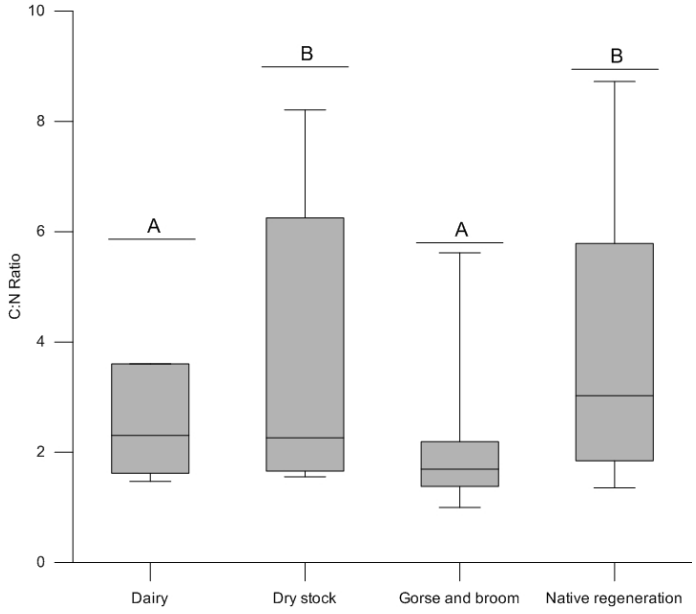

Figure 5.3: effect of land-use on over all resource C:N ratios. Group A had significantly lower C:N rations than groups B (p = 0.041, F = 2.29, n = 31). Whiskers represent upper and lower quartiles

5.4.2 Invertebrate responses and the contribution of basal resource to primary consumers' diets

Collector browsers, filter feeders, grazers and predators were all found within all land-uses. Facilitative shredders were not present in dairy of horticultural streams while shredders were only present within indigenous regeneration streams. Subsequently, shredders were excluded from further analysis. Isotopic mixing model analysis showed that FPOM was the primary trophic resource for lotic invertebrate communities (fig. 5.4), while the contribution of CPOM to community diet was significantly higher than that of biofilm (fig. 5.4).

Surprisingly, land-use had no effect on the diet composition of the pooled communities (P = 0.182, T = 1.42, n = 31). However diet composition of the four primary consumer functional feeding groups did vary significantly with land-use and other environmental drivers (Table 5.3).

Table 5.3: effects of land-use and associated stream parameters on functional feeding group diet composition. Parameter effects are interaction effects with diet resource proportions (i.e. changes in the relative contribution of biofilm, CPOM and FPOM

Diet relationship with:	*df*	*T value*	*P*	*df*	*T value*	*P*
	Collector Browsers			Filter Feeders		
Canopy cover	3	1	0.319	3	2.35	**0.023**
PO_4-P	3	0.49	0.629	3	0.96	0.342
NO_3-N	3	0.77	0.444	3	1.15	0.259
Dissolved Oxygen	3	0.27	0.79	3	1.82	0.075
Land use	8	3.78	**< 0.001**	8	0.93	0.357
	Grazers			Facilitative Shredders		
Canopy cover	3	1.56	0.134	3	1.16	0.263
PO_4-P	3	0.95	0.358	3	1.17	0.255
NO_3-N	3	3.71	**0.002**	3	1.07	0.3
Dissolved Oxygen	3	0.23	0.823	3	1.57	0.127
Land use	8	7.12	0.081	8	0.85	0.42

Not surprisingly, the relative contribution of the three basal resources varied between the four primary consumer functional feeding groups (collector browsers, filter feeders, grazers and facilitative shredders). Across all land-uses, FPOM comprised a larger proportion of filter feeders' diet than all other functional groups ($P = 0.051$) (fig. 5.5). Grazers had the highest proportion of biofilm in their diet ($P = 0.003$). Both collector browsers and facilitative shredders had relatively similar diets which were not significantly different from the community average diet composition.

The diet of collector browsers varied significantly between land-uses. Collector browsers within indigenous regeneration streams feed significantly more on CPOM than those from agricultural streams ($P < 0.001$). Gorse stream collector browsers also had elevated proportions of CPOM within their diet ($P = 0.060$) (fig. 5.6). Filter feeders and facilitative shredders showed no significant effect of land-use on their diet (Table 5.3). There was a weak land-use effect on grazers; populations from dry-stock streams showed lower proportions of CPOM in their diet relative to streams within other land-uses ($P = 0.0814$).

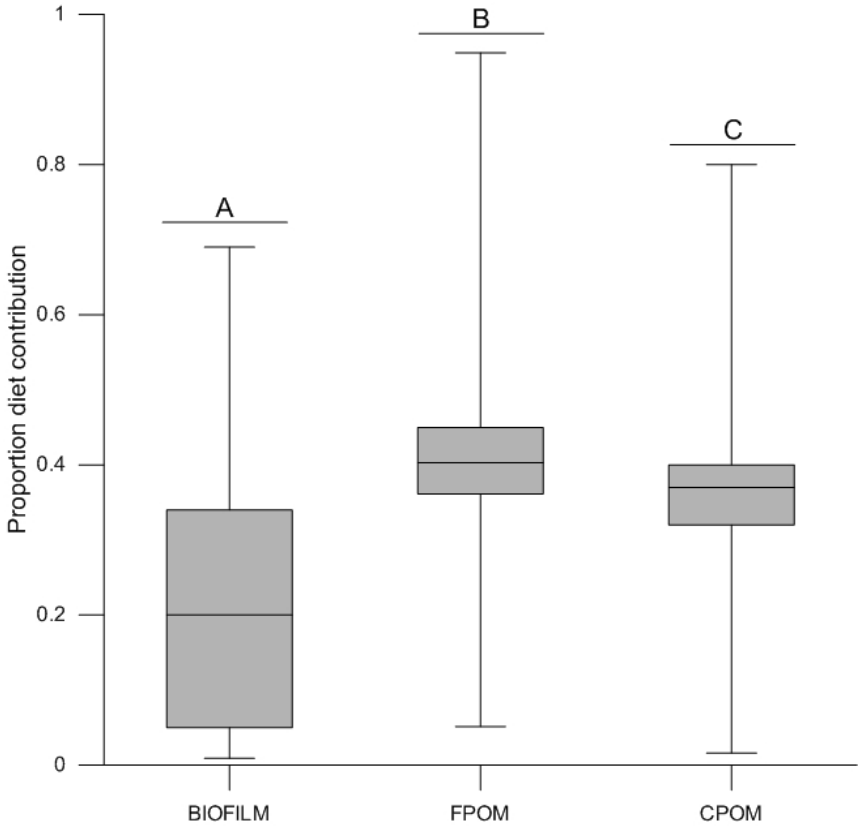

Figure 5.4: average proportional contribution of the three primary trophic sources to macro-invertebrate communities' diet irrespective of land-use. Whiskers present upper and lower quartiles, n = 31

Of the land-use stressors (i.e., NO_3-N, PO_4-P, dissolved oxygen and canopy cover) only nitrate concentration and canopy cover had a significant effect of the diet composition of the primary consumer functional feeding groups (Table 5.3). The proportion of biofilm within the diet of grazers increased with the nitrate concentration of the water (fig. 5.7 A). Filter feeders dietary intake of biofilm increased with light availability (i.e., reduced riparian vegetation cover) (fig. 5.7 B).

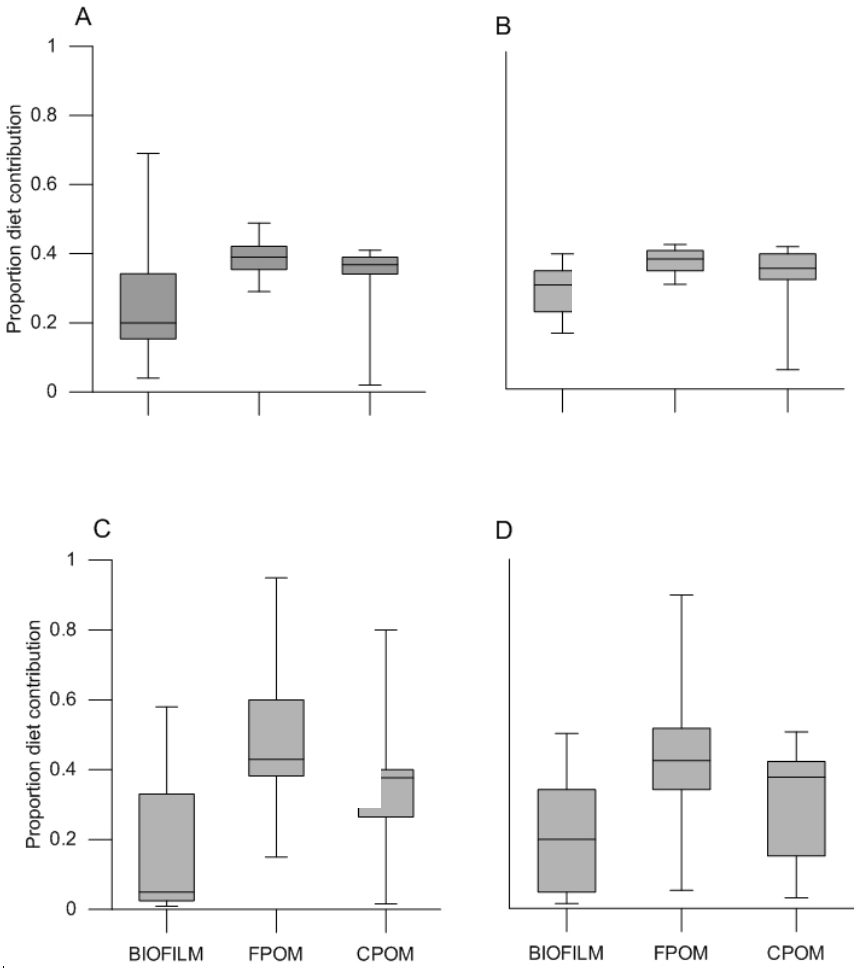

Figure 5.5: dietary source apportionment for the four primary consumer functional feeding groups: A) facilitative shredders, B) grazers, C) filter feeders, D) collector browsers. * denotes where the contribution of a resource differed significantly from whole community averages (refer to Table. 3). Whiskers present upper and lower quartiles. n = 31

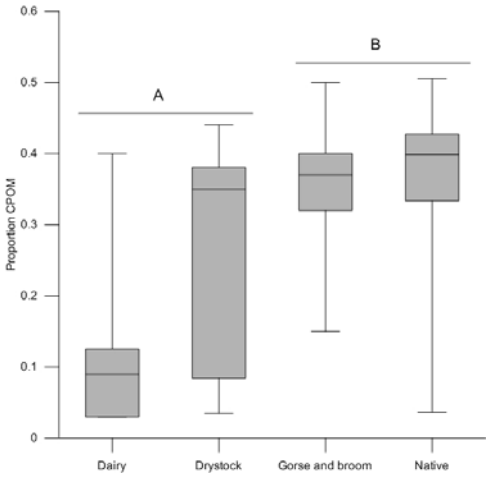

Figure 5.6: contribution of CPOM to collector browsers the four land-use classes. Group A is significantly different from B (refer to table 5.3)

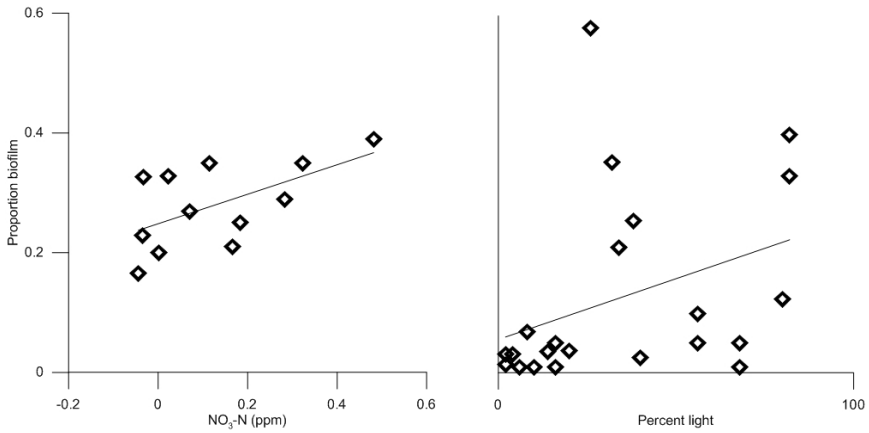

Figure 5.7: relationships between A) proportion biofilm within grazers' diet and stream nitrate-N concentration, r2 = 0.338, P = 0.002, n =12 and B) proportion of biofilm in filter feeders' diet and the amount of light entering the stream (%); r2 = 0.128, P = 0.02

5.4.3 Community-wide trophic responses to land-use

Three community isotopic niche parameters were analysed (i.e., convex hull area, range of $\delta^{15}N$ signatures and the range of $\delta^{13}C$ signatures). The range in $\delta^{13}C$ signatures (C-range) showed the only significant variation between invertebrate communities from different land-uses. There was a significant effect of land-use on the communities C-range ($P = 0.001$) (Table 5.4). Dairy stream communities displayed the largest C-ranges followed by gorse, dry-stock then indigenous regeneration streams respectively (fig. 5.8). When testing the environmental stressors associated with land-use, phosphate-P concentration ($P = 0.008$), dissolved oxygen ($P < 0.001$) and canopy cover ($P = 0.002$) all showed significant relationships with the communities' C-range (Table 5.4). There was also a significant interaction effect between land-use and nitrate-N concentration ($P = 0.023$) on communities' C-range. Phosphate-P concentration and dissolved oxygen both showed negative relationships with C-range while the percent light (inverse of canopy cover) displayed a positive relationship (fig. 5.9). While over all there was no effect of nitrate-N concentration on community C-range when dry-stock and indigenous streams were excluded, there was a negative relationship between nitrate-N concentration on community C-range (fig. 5.9).

Table 5.4: Linear mixed effects model showing relationships of communities' range in δ13C values (C-range) with land-use and associated stressors

Variable	*df*	*Chi Square*	*P*
Land-use	3	15.72	**0.001**
Season	1	0.12	0.839
NO_3	1	1.5	0.213
NO_2	1	0.12	0.839
PO_4	1	7.34	**0.008**
Dissolved Oxygen	1	61.07	**< 0.001**
Canopy cover	1	9.31	**0.002**
Downstream distance	1	0.61	0.419
Land-use x NO3	3	11.32	**0.023**

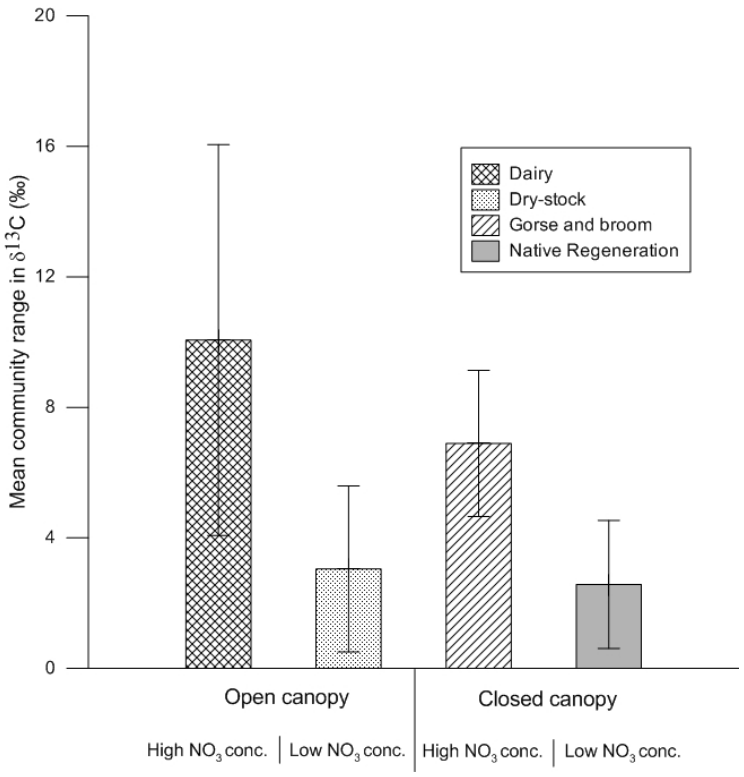

Figure 5.8: Mean range in δ13C values (C-range) in invertebrate communities compared across dairy, dry-stock, gorse and broom and indigenous regeneration stream. Dairy community C-ranges were significantly higher than those in gorse and

broom stream which were significantly higher than those from dry-stock and indigenous regeneration streams (P < 0.001, n = 31). The high and low nitrate-N and open and closed canopy cover classification indicates how the land-uses were initially categorised in the study design. Note however that relationships were actually tested using continuous measures for both nitrate and canopy cover. Error bars = 1 SEM

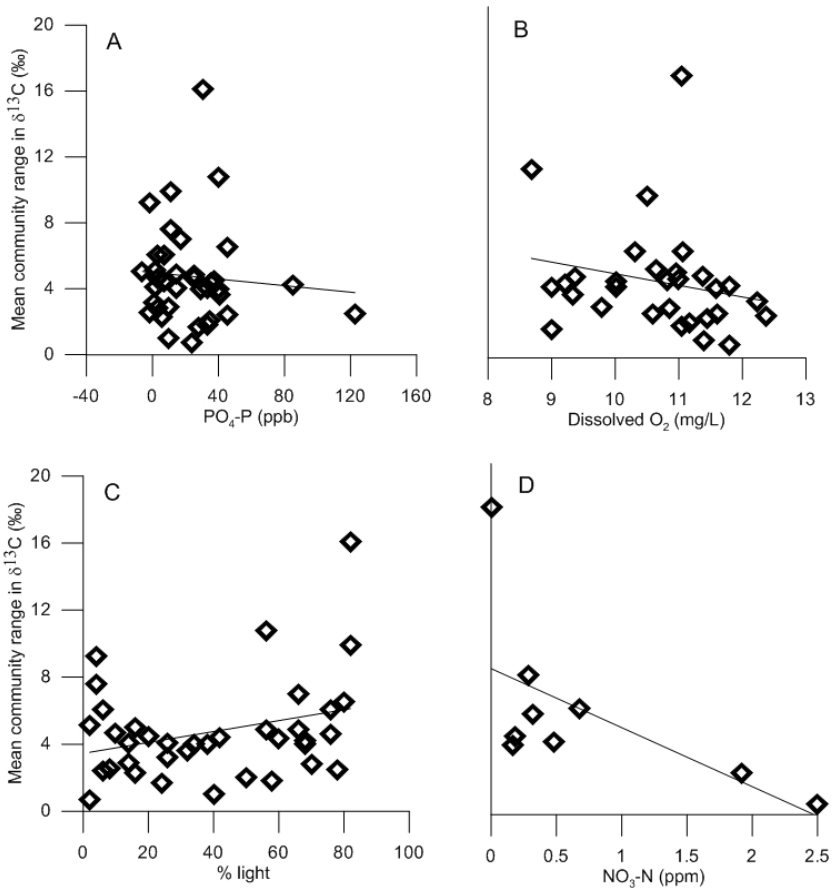

Figure 5.9: relationship between land-use stressors and range in δ13C values (C-range) within lotic invertebrate communities: the stressors are: A) effect of phosphate-P concentration (r2 = 0.010, P = 0.008, n = 31), B) dissolved oxygen (r2 = 0.064, P < 0.001, n = 31), C) percentage light entering the stream (r^2 = 0.100, P = 0.002, n = 31), D) nitrate-N concentration in dairy and gorse stream exclusively (r^2 = 0.367, P = 0.023, n = 9)

5.4.4 Invertebrate $\delta^{15}N$ & $\delta^{13}C$ responses to land-use

Community Invertebrate $\delta^{15}N$ values (i.e. mean $\delta^{15}N$ value for the entire community within a site) were significantly affected by land-use (Table 5.5); invertebrate communities from indigenous regeneration streams had significantly lower $\delta^{15}N$ values than those from all other land-uses (P = 0.003) (fig. 5.10). There was also a significant effect of functional feeding groups (Table 5.5) in which grazers and predators had higher $\delta^{15}N$ values than collector browsers and filter feeders (fig. 5.11). The only other significant effect on invertebrate $\delta^{15}N$ values was canopy cover (Table 5.5); invertebrates within-streams with less canopy cover had higher $\delta^{15}N$ values than those in shades streams. Although there was no significant land-use x functional feeding group interaction effect on invertebrate $\delta^{15}N$ values, the term explained a large portion of the variance (land-use x functional feeding group sums of squares = 445.02 compared the residual sums of squares = 132.09).

None of the predictor variables (land-use and its associated stressors and functional feeding group) showed any significant relationship with invertebrate $\delta^{13}C$ values (Table 5.5). The invertebrate community $\delta^{13}C$ values appeared to be relatively unvarying between land-uses (fig. 5.12). The only significant relationship with invertebrate community $\delta^{13}C$ values was longitudinal downstream distance within the catchment. Invertebrate $\delta^{13}C$ values increased with distance downstream.

Table 5.5: Linear mixed effects model showing the controls of invertebrate $\delta^{15}N$ & $\delta^{13}C$ values

Variable		*df*	*F*	*P*		*df*	*F*	*P*
Land-use	**$\delta^{15}N$**	5	3.28	**0.003**	**$\delta^{13}C$**	5	1.37	0.276
FFG		4	13.97	**< 0.001**		5	0.86	0.558
Season		1	0.01	0.937		1	2.35	0.14
NO3		1	4.69	0.059		1	1.15	0.296
NO2		1	0.001	0.972		1	1.136	0.297
NH4		1	N/A	N/A		1	N/A	N/A
PO4		1	1.09	0.234		1	0.04	0.853
pH		1	N/A	N/A		1	N/A	N/A
Dissolved Oxygen		1	N/A	N/A		1	N/A	N/A
Canopy cover		1	8.05	**0.02**		1	0.08	0.789
Downstream distance		1	0.12	0.737		1	18.15	**0.003**
Land-use x FFG		16	2.28	0.109		16	2.5	0.064

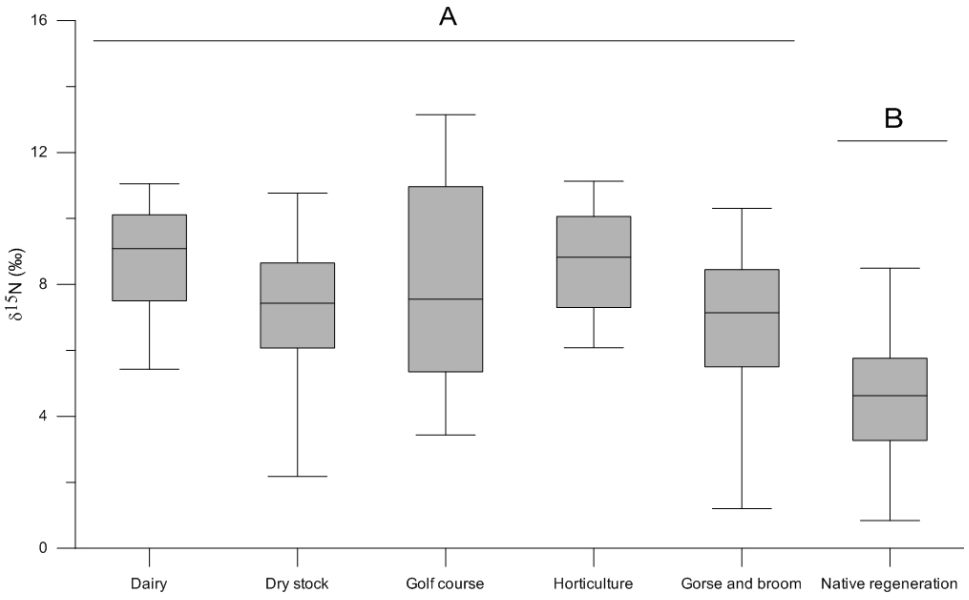

Figure 5.10: average macro-invertebrate δ15N value across the six land-uses. Groups A and B were significantly different (p = 0.003, F = 3.28, n = 35). Whiskers represent upper and lower quartiles

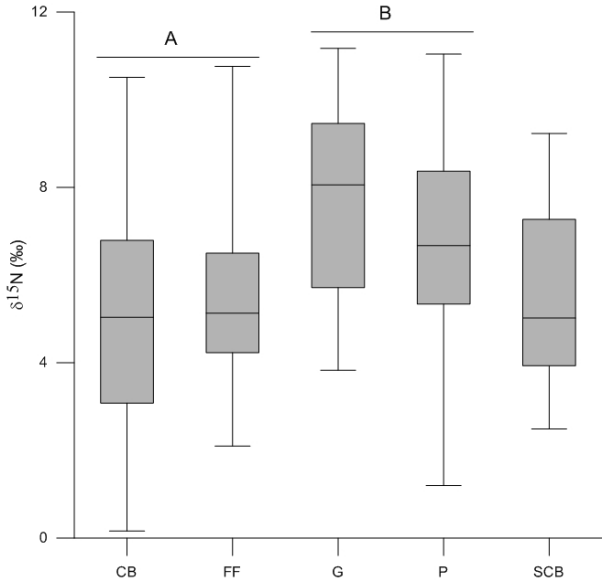

Figure 5.11: average macro-invertebrate δ15N value compared functional feeding groups: collector browsers (CB), filter feeders (FF), grazers (G), predators (P) and facilitative shredders (SCB). Group A had significantly lower δ15N than group B ($p < 0.001$, $F = 13.97$, $n = 35$). Whiskers represent upper and lower quartiles

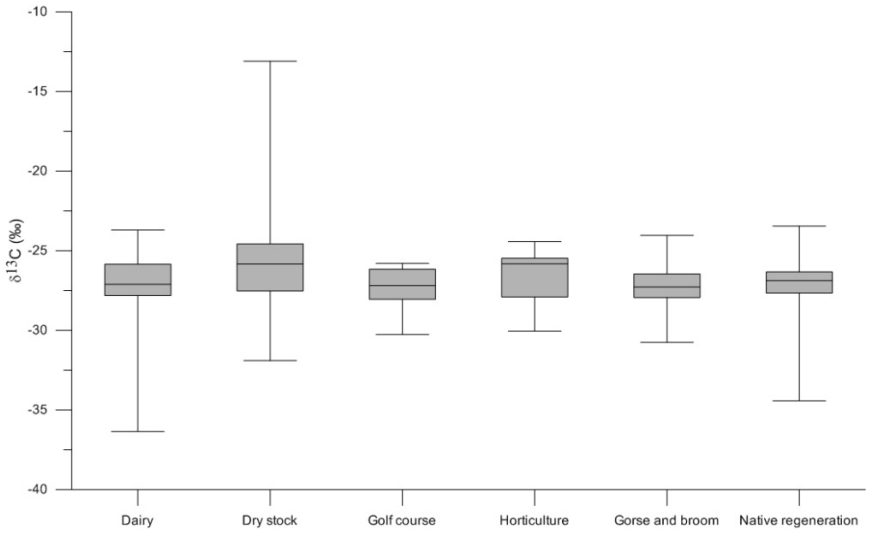

Figure 5.12: average δ13C value of macro-invertebrates compared across the six land-use classes. Whiskers present upper and lower quartiles. n = 35

5.4.5 Functional feeding group $\delta^{15}N$ & $\delta^{13}C$ responses to land-use

Descriptive comparisons were able to be made between functional feeding groups across land-uses (fig. 5.13). Visual comparisons suggest that there were differences in the $\delta^{15}N$ & $\delta^{13}C$ values on functional feeding groups between land-uses which were not detected within the linear mixed effects analysis. Collector browser populations compared between the six land-uses showed the largest variation in both their $\delta^{15}N$ & $\delta^{13}C$ isotopic values of all functional feeding groups (fig. 5.13 A). Filter feeders and predators had the narrowest range in $\delta^{13}C$ isotopic values while both displaying high variation in their $\delta^{15}N$ values (fig. 5.13 B & E). Grazers in all land-uses except for dairy streams had a narrow range of $\delta^{13}C$ isotopic values; dairy stream populations relatively ^{13}C deplete and had the largest associated variation. One interesting patternn observed when comparing functional feeding groups across land-uses was that the two agricultural land-uses (dairy and dry-stock) were consistently more isotopically similar to other land-uses than each other (fig. 5.13).

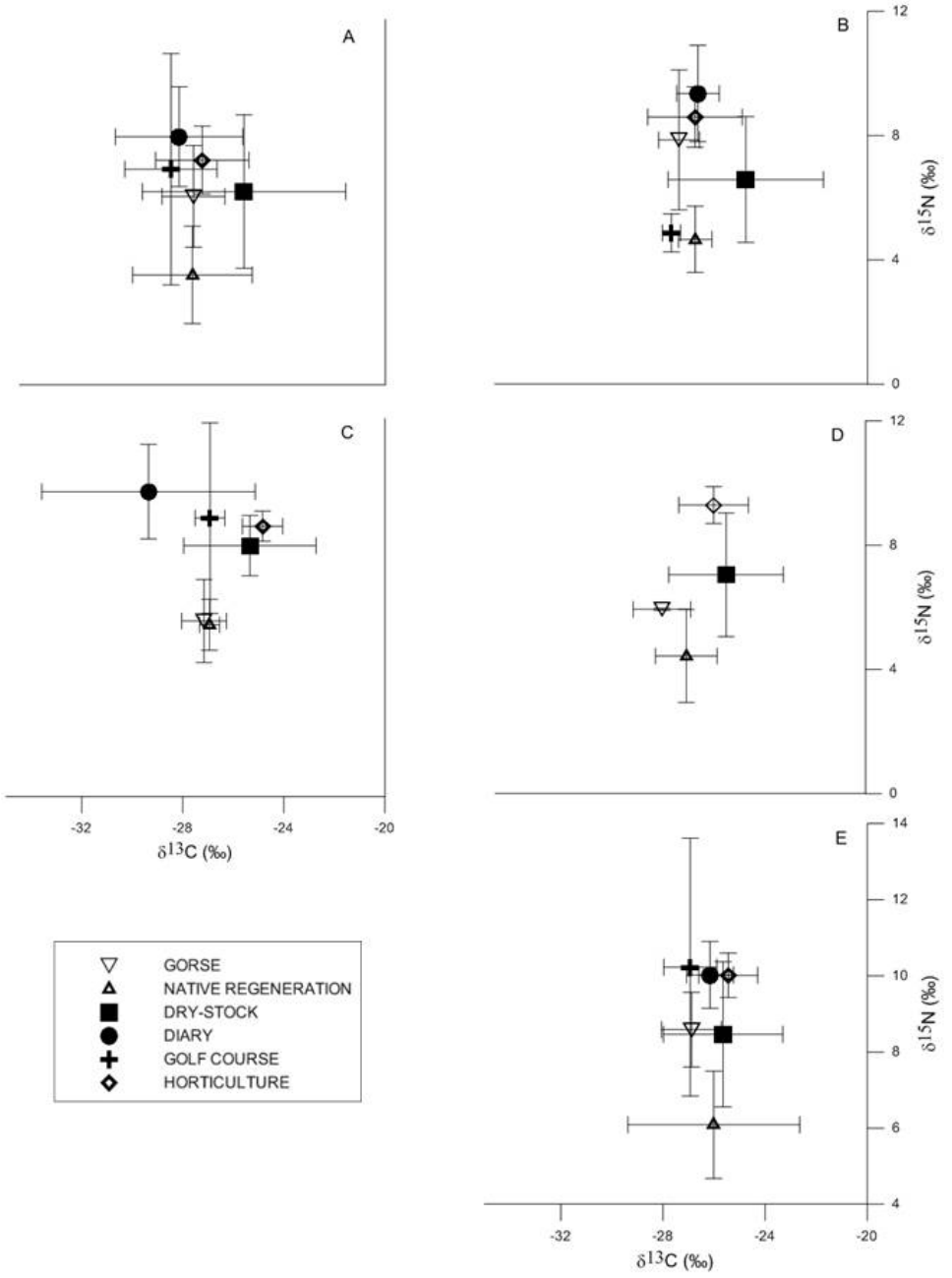

Figure 5.13: invertebrate δ13C & δ15N stable 5.isotopic biplots comparing land-use for five functional feeding groups: A) collector browsers, B) filter feeders, C) grazers, D) facilitative shredders, E) predators. Error bars = 1 SEM. n = 35

5.4.6 Response of common invertebrate taxa $\delta^{15}N$ & $\delta^{13}C$ to land-use

The two most common collector browsers were the mayfly *Deleatidium* and the caddisfly *Pycnocentrodes*. *Deleatidium* were present across all land-uses while *Pycnocentrodes* were found in sufficient numbers in indigenous regeneration, dry stock, horticultural and golf course streams (fig. 5.14). Although *Deleatidium* showed no distinct differences between land-use populations (fig. 5.14 A), *Pycnocentrodes* populations displayed distinct isotopic differences between land-uses. Populations from golf course streams had relatively higher $\delta^{15}N$ values than those from dry stock and horticultural streams while indigenous regeneration populations, $\delta^{15}N$ values were lower again (fig. 5.14 B).

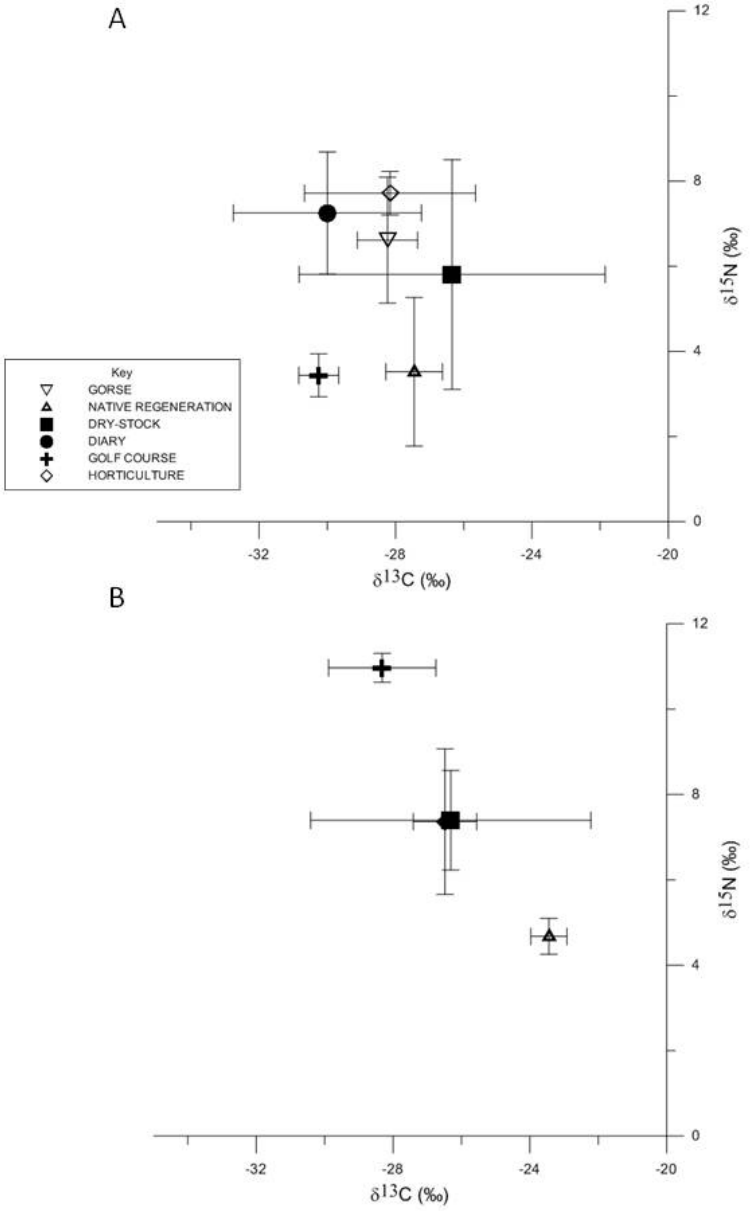

Figure 5.14: mean δ13C and δ15N stable 5.isotopic signature for the two most common collector browser taxa: A) the mayfly *Deleatidium* (n = 31) and B) the caddisfly *Pycnocentrodes* (n = 8) compared across six land-use categories. Error bars = 1 SEM

The most common filter feeding taxa were the mayfly *Coloburiscus* and the net-spinning caddisfly *Aoteapsyche*. *Coloburiscus* were found in sufficient numbers within all land-use streams except for dairy and horticultural while *Aoteapsyche* were represented in all land-uses (fig. 5.15). Both species showed comparable ranges in $\delta^{13}C$ values across land-uses; for both taxa, $\delta^{13}C$ values land-uses followed the same patternn ordered from lowest to highest values (fig. 5.15). When looking at $\delta^{15}N$ values, *Coloburiscus* and *Aoteapsyche* also showed similar values across land-uses (fig. 5.15).

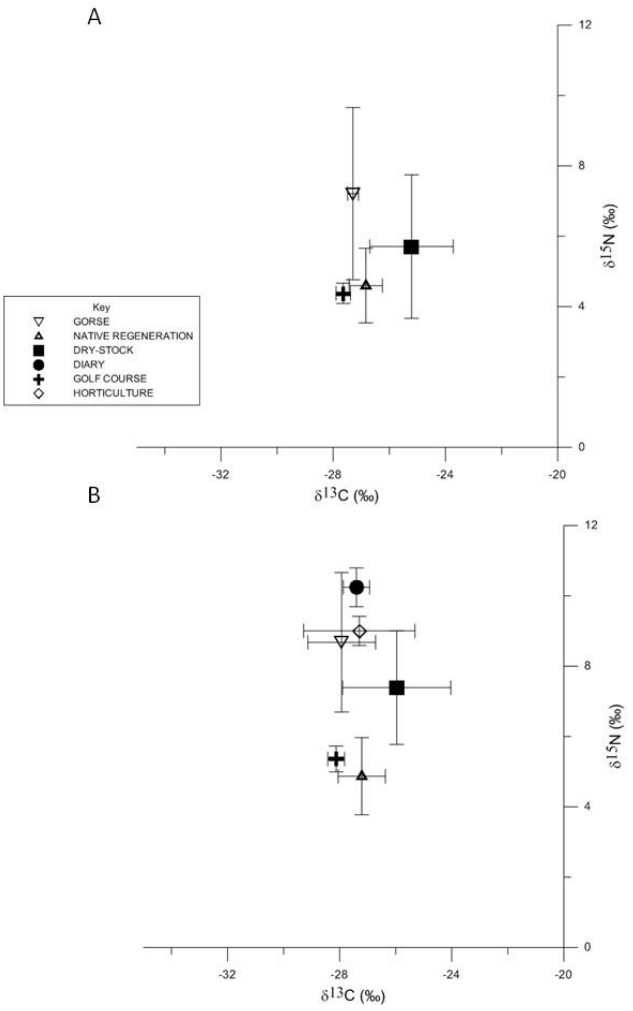

Figure 5.15: mean δ13C and δ15N stable 5.isotopic signature for filter feeder taxa: A) the mayfly *Coloburiscus* (n = 19) and B) the net-spinning caddisfly *Aoteapsyche* (n = 21) compared across six land-use categories. Error bars = 1 SEM

The only grazer taxa which was in sufficient number in more than two land-uses was the snail *Potamopyrgus*; it was found in represented in all land-uses except for indigenous regeneration streams. Compared to land-use comparisons at the functional feeding group level, *Potamopyrgus* populations

showed a narrow range in $\delta^{13}C$ values (fig. 5.16). *Potamopyrgus* within dairy streams displayed a similar $\delta^{13}C$ values to other land-use communities than those of combined grazer measurements. Differences in $\delta^{15}N$ values of land-use populations of *Potamopyrgus* were more pronounced than when comparing grazers.

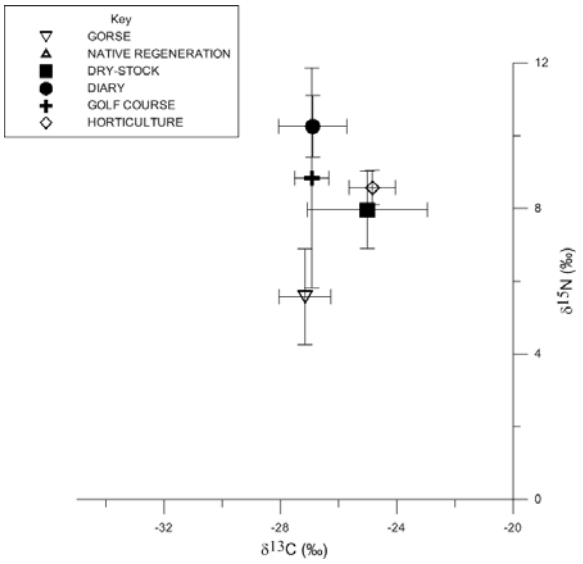

Figure 5.16: mean δ13C and δ15N stable 5.isotopic signature for the grazing snail, *Potamopyrgus*, compared across six tested land-use categories. Error bars = 1 SEM. n = 10

The most common predator, the dobsonfly *Archichauliodes*, was present within indigenous regeneration, gorse, dry-stock and horticultural streams in replicable quantities. The second most abundant predator group was the caddisfly family Hydrobiosidae which were represented within indigenous regeneration, gorse, dry-stock, dairy and horticultural land-uses streams (fig. 5.17).

Archichauliodes populations showed very similar $\delta^{13}C$ values across land-uses with low variability except for indigenous regeneration streams where variance was substantially higher (fig. 5.17 A). Conversely Hydrobiosidae showed differences between land-use populations in regard to $\delta^{13}C$ values; gorse and broom and dairy populations displayed distinct differences in their $\delta^{13}C$ values (fig. 5.17 B). Both showed similar land-use patternns in their $\delta^{15}N$ values in which indigenous regeneration populations had distinctively lower $\delta^{15}N$ values than all other land-uses except for dry stock streams (fig. 5.17).

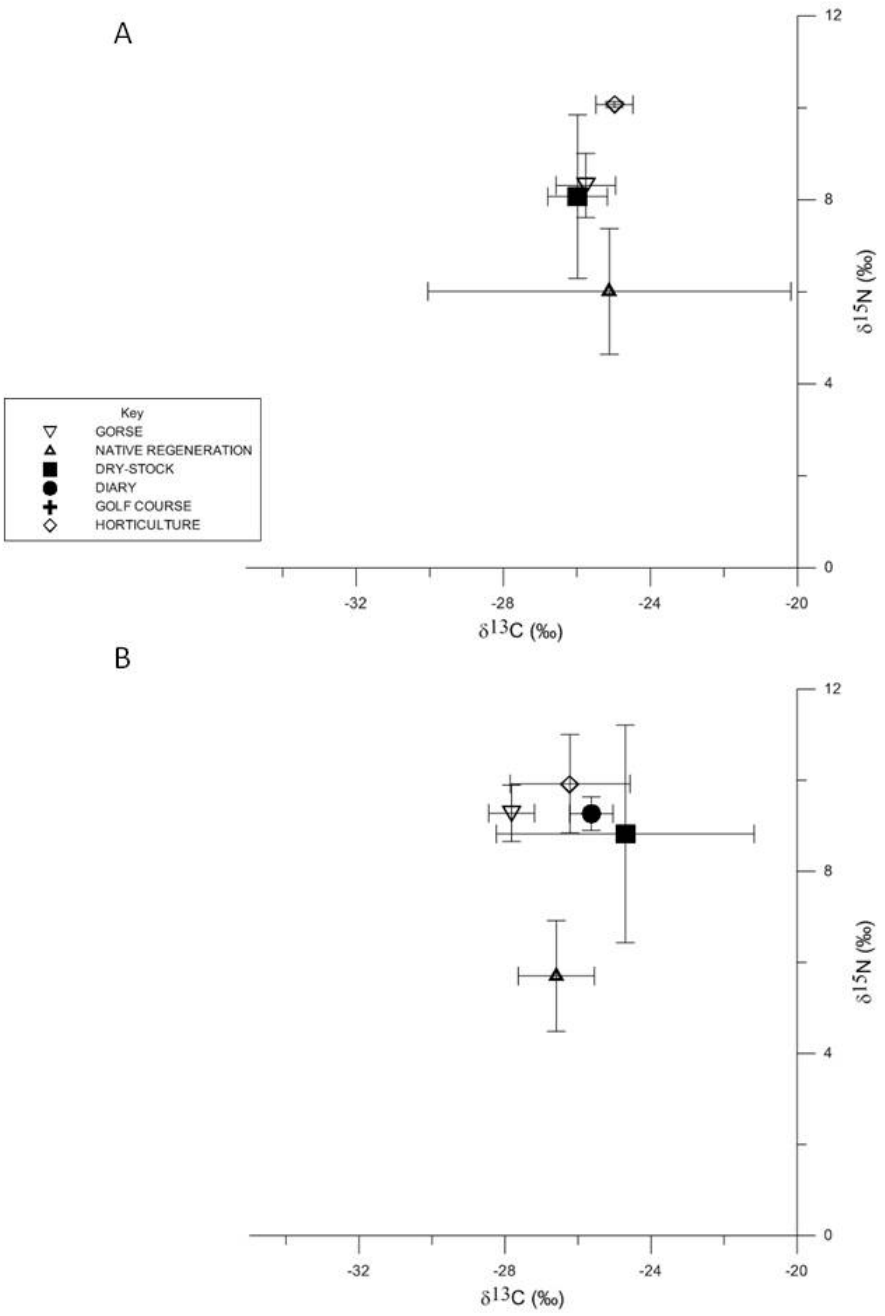

Figure 5.17: mean δ13C and δ15N stable 5.isotopic signature for A: the dobsonfly *Archichauliodes* (n = 18) and B: the caddisfly Hydrobiosidae (n = 11) compared across six tested land-use categories

5.5 Discussion

Overall collector browser invertebrates within regeneration indigenous forest sites consumer more CPOM than those from all other land-uses. At the community level, regeneration indigenous forest communities had a smaller trophic niche than those from gorse and broom and dairy sites. This indicated that land-use was in fact changing the way in which communities were processing organic material. However no consistent pattern with individual stressors was observed at either the inter or intra community levels, suggesting that multiple stressors were in fact interacting. The mechanisms controlling this are discussed below.

Although the average basal resource C:N ratios were lower within dairy and gorse and broom streams than those from indigenous regeneration and dry stock streams, the C:N ratios of the resources relative to each other did not change between land-uses. Across all land-uses CPOM had the highest C:N ratios of the three basal trophic resources, while FPOM and biofilm were relatively similar in regards to C:N. This is consistent with other published studies which indicate that CPOM is considered nutritionally poor and primary producers have been shown to display stoichiometric plasticity (Frost et al. 2005; Hall et al. 2005: Ventrua et al. 2008). However, research suggests that stoiciometric responses will be more pronounced in autochthonous resources than allochthonous (Elser et al. 2000; Hall et al. 2005; Marcarelli et al. 2011). This is because autotrophs will absorb more nitrate-N to produce chlorophyll (Frost et al. 2005). However, CPOM supports colonies of decomposing fungi and bacteria which are stimulated by nutrient availability (Benstead et al. 2009). Although there was no observed relationship C:N ratios and stream nitrate-N concentration, reduced CPOM C:N within dairy and gorse and broom

streams is likely caused by nutrient availability increasing the biomass of fungal and bacterial decomposers within the CPOM.

Despite biofilm having the lowest over all C:N ratio, FPOM was one the most important trophic resources to communities as a whole irrespective of land-use. Harding et al (1997) shown that Banks Peninsula streams communities have a higher proportion of filter feeders than other New Zealand ecoregions. This suggests that supply of FPOM is playing a role in determining trophic interactions within communities on the Banks Peninsula. Across all land-uses, filter feeders had the highest proportion of FPOM in their diet and grazers had the highest proportion of biofilm in theirs. This strong dietary relationship of filter feeders and grazers with their assumed primary resources is in no way a new finding; but it does give validation to other more ambiguous patternns shown within the community.

Based on isotopic mixing model analysis, collector browsers were the only functional feeding group which showed some change in their diet between land-uses. This suggests that irrespective of nitrate supply, collector browsers will opportunistically increase their intake of CPOM when that resource is readily available (e.g. in forested systems). Some invertebrates feed on the microbial decomposers within the CPOM (Benstead et al. 2009). CPOM has been shown to be a limiting resource for invertebrates in open canopy systems (Leberfinger et al. 2011); while increased nitrate availability leads to more rapid processing of CPOM and reduced standing stocks (Benstead et al. 2009). Although this provides a mechanistic answer for why a generalist primary consumer (collector browsers) are consuming more CPOM in indigenous and gorse and broom systems, these drivers (nitrate-N and canopy cover) showed no relationships with CPOM intake, implying that the relationship is more complicated.

Stressors associated with land-use showed differing trophic responses within functional feeding groups. The proportion of biofilm in the diets of grazers and filter feeders increased with nitrate and light stress respectively. Light and nitrate-N represent two primary resources limiting primary production in lotic systems (Dodds 2007). Increasing these resources would be expected to lead to an increase in biofilm production (of which algae is a significant component). While biofilm was commonly only a small component of primary consumers' diets, its high nutritional quality means that small changes in its dietary contribution can be a measurable indicator of increased algal production. This is important since relationships between nutrient additions and increases in algal production can be masked by high turnover rates (Sandin and Solimini 2009). It also highlights that drivers of consumer – resource relationships can differ substantially different functional groups. When multiple stresses, and responses, are associated with a land-use the combination can result in emergent community-wide land-use patternns (Singer and Batten 2007).

Carbon-range provided an integrated community trophic response to land-use. Communities within dairy streams had the largest C-range followed by gorse, dry-stock and indigenous regeneration respectively. This suggests that communities within indigenous regeneration streams are supported by fewer trophic pathways than in communities in other land-uses. Wider trophic niches (i.e. C-range) are traditionally considered indicative of increased food web stability (McCann et al. 2005; Layman et al. 2007). A similar positive relationship between disturbance intensity (invasive macrophytes) and C-range of fish and odonates has hypothesised by Kovalenko and Dibble (2011). They suggest this relationship was driven by a decrease in first choice trophic resources resulting in consumers foraging over greater distances and thus encountering a wider variety of trophic resources. Potentially, the increased amount of CPOM within indigenous regeneration streams provides favourable habitat for invertebrates

The mechanisms by which the individual land-use stressors affected this overall relationship between land-use and the increased C-range are obviously complex as indicated by the multiple significant stressor effects and interactions. Relationships with C-range were found for dissolved oxygen, phosphate concentration, canopy cover and nitrate concentration. All of these stressors are also important parameters of ecosystems metabolism (Dodds 2007). This suggests that whole community trophic responses to land-use are by in large driven by processes which impact production on basal resources as opposed to factors limiting consumers' ability to utilise the available resources.

$\delta^{13}C$ stable isotopic niche indices were larger at low phosphate-P concentrations, indicating that communities were feeding on a wider range on resources when phosphate concentrations were low. Trophic niches were also larger when dissolved oxygen concentrations were low and light availability was high. Nitrate-N concentration decreased in the community carbon range only within dairy and gorse streams. The positive relationship between light and C-range shows that feeding is in part driven by increased autochthonous production (Leberfinger et al. 2011), and is conducive with patternns seen between land-uses. However, the relationships for phosphate-P and nitrate-N with C-range were somewhat contradictory to the overall land-use effect in which C-ranges were smallest in indigenous regeneration sites (i.e. low nitrate-N and phosphate-P concentrations). Interestingly the negative C-range – nitrate-N relationship was only significant in high nitrate-N concentration land-uses, suggesting that nutrient effects are more pronounced at higher levels. Using these land-use stressor relationships to account for the patternns observed between land-use and C-range will likely require investigating interactions between these and other stressors (i.e. sedimentation) (Matthaei et al. 2006). While I lacked the statistical power to test for multiple interactions between these terms, the discrepancies between the effects of drivers and overall land-use suggests that such interactions are indeed occurring.

Comparing C-ranges of lotic invertebrate communities from differing land-uses is potentially a powerful tool for assessing land-use impacts.

Although the diet of collector browsers, filter feeders and grazers varied between land-uses, this did not result in isotopic differences in functional feeding groups. There was no difference in both $\delta^{13}C$ and $\delta^{15}N$ values of any of the functional feeding groups from different land-uses. This suggests that dietary changes cannot be identified within invertebrate communities without basal resources' isotopic values and the subsequent use of isotopic mixing models. Instead the $\delta^{15}N$ values of whole invertebrate communities were lower in indigenous regeneration streams than all other land-uses. Grazers and predators also had higher $\delta^{15}N$ values than all other functional feeding groups regardless of land-use. This further supports the idea that land-use effects are more strongly represented at the whole community level rather than between functional feeding groups. High predator $\delta^{15}N$ values suggests that they are feeding at higher trophic level than other functional feeding groups (Post 2002); however we know that grazers are feeding primarily on biofilm. This indicates that autochthonous production has higher $\delta^{15}N$ values than allochthonous. No land-use and functional feeding group effects were seen at all in $\delta^{13}C$ values. Variation in invertebrate $\delta^{15}N$ values without significant changes in their $\delta^{13}C$ values suggests that nitrogen within biofilm is isotopically distinct between indigenous regeneration and all other land-use streams. Elevated $\delta^{15}N$ values have been used as an indicator of land-use induced disturbances to nitrogen cycling (Robinson 2001; Diebel and Vander Zaden 2008; Clapcott et al. 2011). While this implies that nitrogen cycling is affected by all anthropogenic land-uses, it does not necessarily mean they are all impacted in the same way. Various processes within the nitrogen cycle can cause $\delta^{15}N$ enrichment (e.g. ammonia volatilisation, denitrification autotrophic uptake) (Robinson 2001; Stevenson et al. 2010). Although detrital trophic pathways do respond to nitrate additions (e.g. Benstead et al.

2009), my results suggest that autochthonous resources (and consumers feeding on them) are a better indicator of land-use affects than allochthonous ones.

While a statistical comparison was not possible, comparing the response of invertebrate taxa to land-use appeared to reveal isotopic differences which were masked within functional feeding groups. This was most pronounced when comparing the collector browser caddisfly *Pyconcentrodes*, the grazing snail *Potamopyrgus* and the predatory dobsonfly *Archichauliodes*. *Pyconcentrodes* showed three isotopically distinct groups; those from golf course streams, those from horticultural and dry-stock streams and those within indigenous regeneration streams. This is strong evidence for land-use induced diet changes. *Pyconcentrodes* has been identified as a generalist feeder which will switch trophic resources based on nutritional quality (Ledger and Winterbourn 2000). Conversely, *Potamopyrgus* and *Archichauliodes* displayed far less variation in their $\delta^{13}C$ values than their respective functional feeding groups did across land-uses. *Archichauliodes* are a generalist predator (Devonport and Winterbourn 1976), meaning it is likely that their $\delta^{13}C$ values represent an average of the whole primary consumer community. This would result in less isotopic variation (Flaherty and Ben-David 2010). Conversely, *Potamopyrgus* will feed exclusively on biofilm if given the chance (Winterbourn 2004); thus their narrow range of $\delta^{13}C$ values is likely to be due to diet specialization. This would make them a good indicator taxa for investigating responses of biofilm production to land-use stressors. While stable isotopic analysis of invertebrate taxa presents the opportunity for a more fine detail investigation of land-use impacts on lotic invertebrate communities, only one taxon (the mayfly *Deleatidium*) was present in all six land-uses. The absence of taxa in land-uses (while important in itself) would prevent a full land-use comparison.

5.6 Conclusion

While we intuitively know that anthropogenic land-uses impact lotic ecosystems, due to the presence of multiple interacting stressors, quantifying these impacts can be problematic. $\delta^{13}C$ and $\delta^{15}N$ stable isotopic analyses of lotic invertebrate communities can be a powerful tool for understanding these impacts. Through investigating invertebrate community responses at inter-community and intra-community levels, I have shown that land-use can drastically change the way in which nitrogen and carbon (i.e. organic material) is moving through these communities. However, there was no clear relationship as to which land-use had the most impact on invertebrate communities, which justifies investigating multiple community trophic responses. The reduced CPOM consumption and narrower trophic niches observed in collector browsers in all land-uses relative to indigenous regeneration sites indicates that the presence of CPOM is a strong driver of trophic interactions. However, while grazers and filter feeders showed no relationships between land-use *per se* and their diet, both increased their biofilm intake with nitrate and light availability respectively. Again the relationships between community trophic niche (carbon range) and individual land-use stressors (dissolved oxygen, phosphate-P concentration, nitrate-N concentration and light availability) displayed a variety of complex patternns. When looking at these results collectively, they demonstrate that the way in which an invertebrate community responds to changes in nitrate availability and cycling will depend on other land-use stressors. This may have significant impacts on the routing of vital nutrients, such as nitrogen, demonstrating that it is important to account for changes in multiple community trophic attributes when quantifying investigating land-use impacts.

Comparing stable isotopic values of invertebrates showed significant relationships between the $\delta^{15}N$ values of functional feeding groups, in that grazers and predators had higher $\delta^{15}N$ values across all land-uses. Furthermore, communities within all five human impacted land-uses had higher $\delta^{15}N$ values

than indigenous regeneration stream communities. However dietary changes of functional feeding groups which were shown using isotope mixing models were not detected in the actual $\delta^{13}C$ and $\delta^{15}N$ stable isotopic values of the invertebrates. This suggests that $\delta^{15}N$ stable isotopic values of whole lotic communities may present a useful indicator for land-use induced effects on nitrogen cycling.

While investigating individual taxa presents the possibility of better detecting dietary shifts and nitrogen cycling impacts, overall my results show that using community-wide niche measures and comparisons of functional feeding group diet composition are nonetheless a powerful method for detecting food web and ecosystem responses to land-use. With and increasing human population, and once which is increasing in affluence, land-use effects are going to continue to be a major management challenge through the 21st century. While it is hard to say if one land-use is worse than another, being able to measure effects in holistic fashion which can account for food web and ecosystem responses is a powerful management tool. Further studies investigating stable isotopic relationships across wider land-use gradients and integrating more stressors will greatly increase their applicability and result in a powerful future management tool for lotic systems.

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Chapter 6: Summary

6.1 Overview of findings

As indicated in chapter two, the relationship between land-use and the impacts on stream ecosystems is a highly complex. This is specifically emphasized when focusing on the causes and effects of elevated nitrate. Nonetheless, data presented in chapters four and five show that by using multiple stable isotopic and other environmental proxies significant insight can be gained into these complex relationships.

The relationship between gorse and broom land-use and high nitrate-N concentrations (between 0.17 and 2.51 ppm) identified a regional nitrate source which was previously unidentified. However, the nitrate – $\delta^{15}N$ & $\delta^{18}O$ data provided strong evidence that this relationship was not a land-use legacy effect but was in fact due to nitrogen which was fixed by the gorse and broom plants themselves. Overall, all anthropogenic land-uses displayed elevated nitrate levels (albeit non-significant) relative to native regenerating forest systems as well as higher nitrate – $\delta^{15}N$ values. Furthermore, nitrate – $\delta^{15}N$ & $\delta^{18}O$ values covaried, indicative of biological fractionation, within all anthropogenic land-uses (except for horticultural sites which had insufficient replication to test for a relationship) but not within native regenerating forest sites. Relationships between nitrate – $\delta^{15}N$ & $\delta^{18}O$ values and physicochemical parameters suggested that nitrogen cycling occurred more efficiently and localized within native regeneration sites. Within gorse and broom systems the relationships between nitrate – $\delta^{15}N$ values and physicochemical parameters suggested that this biological fractionation was primarily driven by autotrophic and heterotrophic nitrogen uptake; however, whether denitrification and uptake was the primary nitrate removal mechanism was less clear within dry stock, dairy and golf course streams. The physicochemical parameters which best explained nitrate – $\delta^{15}N$ & $\delta^{18}O$ fractionation relationships

varied substantially between land-uses, indicating that nitrogen cycling was affected in differing ways across land-uses.

When investigating how these land-use impacts manifested within invertebrate communities at the level of functional feeding groups, collector browsers were observed to feed on less CPOM in gorse, dry stock and dairy sites relative to native regeneration sites. Grazers also increased the proportion of biofilm in their diet with increasing nitrate concentration. These dietary changes however, did not manifest in significant differences between the average stable isotopic signatures of invertebrate functional feeding groups between land-uses. When using isotopic niches to investigating whole invertebrate community trophic niche responses a significant land-use effect was observed. The invertebrate community carbon range in dairy streams was significantly larger than in gorse and broom streams while in dry stock and native regeneration stream communities the niche was smaller than gorse and broom and dairy streams. However the relationships between individual physicochemical parameters and community carbon range did not show any clear patternn as to what was primarily driving this land-use relationship. When comparing average $\delta^{15}N$ values of the whole community between land-uses native regeneration sites had lower values than all other land-uses. Over all, this suggested that community-wide measures were best at describing general land-use effects.

6.2 Integration of findings

At first these two lines of research may seem disparate; however, they are in fact proximate and ultimate measures of the same relationship and are highly complementary. The fact that, at least within gorse and broom streams, nitrate removal appeared to be primarily driven by autotrophic and heterotrophic uptake means that this assimilated nitrogen is becoming available as trophic resources for the invertebrate community. This suggests that factors impacting the uptake of nitrate should thus have

direct consequences on trophic interactions within the foodweb. Such relationship was shown through the proportion of biofilm consumed by grazers increasing with nitrate concentration. However, no other direct relationship between nitrate concentration and trophic interactions were observed within stream communities. Invertebrate community diet was impacted by overall land-use however. Rather than suggesting that no relations exists between nitrate uptake and foodweb trophic interactions within invertebrate community members other than grazers, it highlights the complexities introduced through interactions associated with other land-use associated stressors (Galloway et al. 2003; Allen 2004; Dodds 2007). When comparing both physicochemical relationships between nitrate – $\delta^{15}N$ & $\delta^{18}O$ values and between physicochemical land-use stressors and invertebrate $\delta^{15}N$ values, it was apparent at both levels that environmental conditions beyond the supply of nitrate were impacting observed isotopic values.

It is a fair assumption that grazers will have the strongest relationship with biofilm as they tend to have dedicated mouth-parts for scrapping algae fro rocks (Winterbourn 2004). However, elevated nitrate levels have been previously shown to impact breakdown of CPOM and export of FPOM (Benstead et al. 2009; Harner et al. 2009). The ways through which land-use change can impact nitrogen cycling and trophic interactions within the lotic community are wide ranging. When comparing land-uses, the average $\delta^{15}N$ values of invertebrate communities appeared to follow the patternn observed with nitrate $\delta^{15}N$ values. This suggests that impacts with the nitrogen cycle are being recorded within the $\delta^{15}N$ values of invertebrate consumers. $\delta^{15}N$ values have been used as an integrated measures of land-use impacts on nitrogen cycling has been within lotic and soil systems previously (Diebel and Vander Zaden 2008; Clapcott et al. 2010; Stevenson et al. 2010). The primary against the use of $\delta^{15}N$ values as an integrated measure of nitrogen cycling is that they are unable to distinguish between

multiple processes which result in high $\delta^{15}N$ values (Robinson 2001). However, my findings show that a more mechanistic understanding can be achieved when multiple proxies are measured. Relationships within nitrate – $\delta^{15}N$ & $\delta^{18}O$ can provide evidence of the mechanisms acting within the nitrogen cycle while investigating diet apportionment and trophic niches can reveal where within the lotic community these impacts can best be detected. Bringing these components together provides valuable insight into both long trends and immediate responses to land-uses impacts in nitrogen cycling.

Reducing some of the variation associated with invertebrate $\delta^{15}N$ responses to land-use and ultimately nitrogen-cycling processes may be achieved through focusing on specific taxa. As mentioned grazers, in particular the ubiquitous snail *Potamopyrgus* may be a strong indicator of autotrophic responses to land-use change which warrants further investigation; while the filter feeding mayfly *Coloburiscus* and the collector browser mayfly *Deleatidium* may indicate responses within FPOB and CPOM respectively. Nonetheless, these will only provide proxies in the absence of nitrate – $\delta^{15}N$ & $\delta^{18}O$ data and I strongly recommend using multiple stable isotopic proxies coupled with other measures of environmental parameters to elucidate land-use impacts on nitrogen-cycling and community responses.

An emergent property which was brought to light several times during this research was the interactions of multiple elemental cycles with the nitrogen cycle; namely phosphorous and carbon. Interactions between these cycles have been widely noted but sparsely studied (Dodds 2007; Gruber and Galloway 2008). Phosphate concentration had a significant effect on nitrate uptake within gorse and broom streams also on the community carbon range across all land-uses. Nitrogen and phosphorous are the primary limiting nutrients within most ecosystems; thus the availability of one will impact the ecosystems' response to the other (Dodds et al 2007). Moving beyond accounting for phosphate availability when studying nitrogen cycling land-use effects to quantifying how these cycles interact will

be a significant advance in understanding land-use impacts on stream ecosystems. Similarly, nitrate uptake and community trophic interactions were impacted by the availability of both autochthonous (i.e. sunlight) and allochthonous carbon. As carbon is the primary unit of metabolic energy, it is also inherently coupled with phosphorous and nitrogen cycles. With future land-use intensification and the expected increase in nutrient fluxes (primarily nitrogen), these cycles are expected to become less tightly coupled (Gruber and Galloway 2008). Changes in the interactions between these three elements are likely to impact the nutritional quality of trophic resources and impact which environments act as carbon sources and sinks. While these issues were not addressed here specifically, the trends within the data highlight the future need to address them.

6. 3 Conclusion

We are in an era where land-use changes are occurring rapidly and frequently. It can be difficult for the science and monitoring of associated impacts on stream ecosystems to keep track with these changes. Over the last century important biodiversity and ecosystem functions have been drastically altered without any record of baseline conditions both within New Zealand and around the world. Having analytical tools which allow for rapid and detailed interpretation of the processes occurring within an ecosystem and their response to land-use is an imperative objective. Here I have shown that stable isotopic analyses con provide a robust foundation to such an analysis. Using this I have identified a previously unidentified nitrate source and demonstrated land-use specific responses in invertebrate community trophic interactions. This warrants further investigation into the use of stable isotopic analyses for describing and quantifying the impacts of land-use on a vital ecosystem function, nitrogen cycling. In New Zealand this research is still in its infancy and will greatly benefit from more wide spread data collection. I hope the data collected in this study will become a commons resource available to

future studies. The challenges faced by environmental practitioners in the 21st century will be immense; but with effective tools and cooperation, it is a challenge which can be met.

6.4 Literature cited

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Appendix 1: Measure of the degree of scatter observed within nitrate $\delta^{18}O$ & $\delta^{15}N$ values using convex hull areas

In order to determine relative clustering of land-use nitrate stable isotopic values, convex hull polygons were fitted around nitrate $\delta^{18}O$ & $\delta^{15}N$ values within isotopic space. Commonly applied with stable isotopic foodweb studies (Layman et al. 2007), convex hulls provide a quantitative, integrated measure of the range in both $\delta^{18}O$ & $\delta^{15}N$ values associated with nitrate from a land-use. Areas were obtained using the the analytical graphics packages “splancs” and “grDevices” within R

Figure app. 1 shows the polygon areas and densities for nitrate values within isotopic space. The significance of the single most enriched sample was apparent through comparing the dairy polygons with the data point included and excluded; dairy isotopic values displayed the lowest density. Dairy streams had the largest polygon area when all data points were included. Dry stock streams had the next lowest density was followed by golf course, native regeneration, gorse and broom and horticultural streams respectively. The imbalanced sample sizes made it hard to make firm statements on the relative clustering of nitrate isotopic values for the different land uses. This was highlighted by native regeneration streams occupying a large area in isotopic space but having a higher density than golf course nitrate isotope values due to differences in sample size (fig. 4).

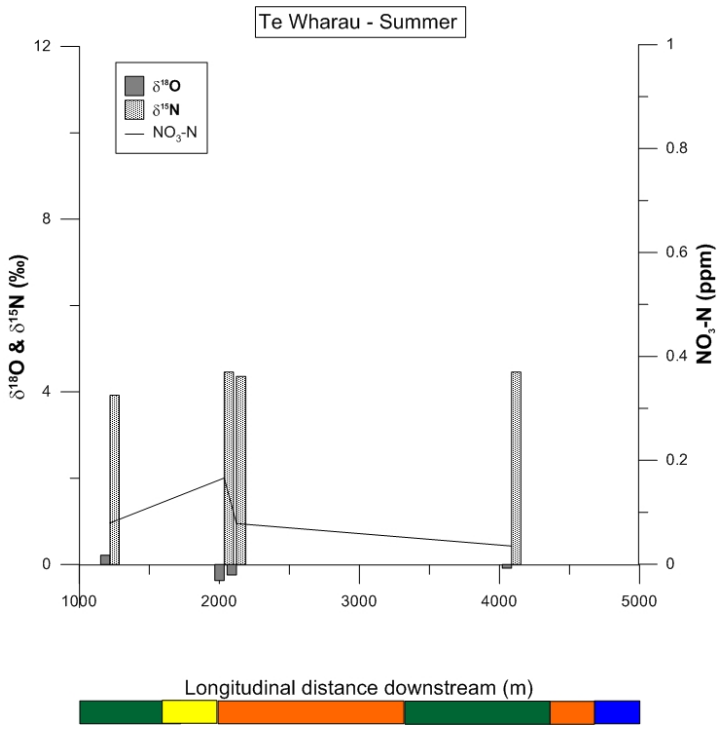
Figure app. 1: nitrate isotopic value polygon areas. Legend indicates the isotopic area occupied by each land-uses isotopic values as well as n (number of values recorded) and density (number of values within polygon). Two dairy polygons were included to highlight the effect of including and excluding the most enriched isotopic value. Lime green = dairy, red = horticulture, yellow = gorse and broom, forest green = indigenous regeneration, blue = golf course and orange = dry stock.

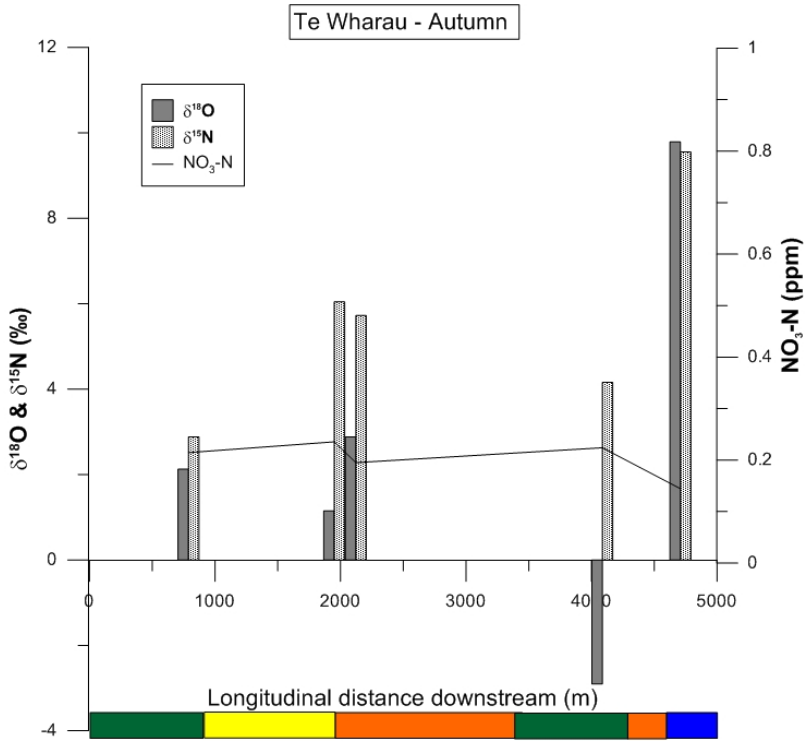
Appendix 2: Longitudinal patternns within nitrate $\delta^{18}O$ & $\delta^{15}N$ values and nitrate-N concentrations down catchment lengths

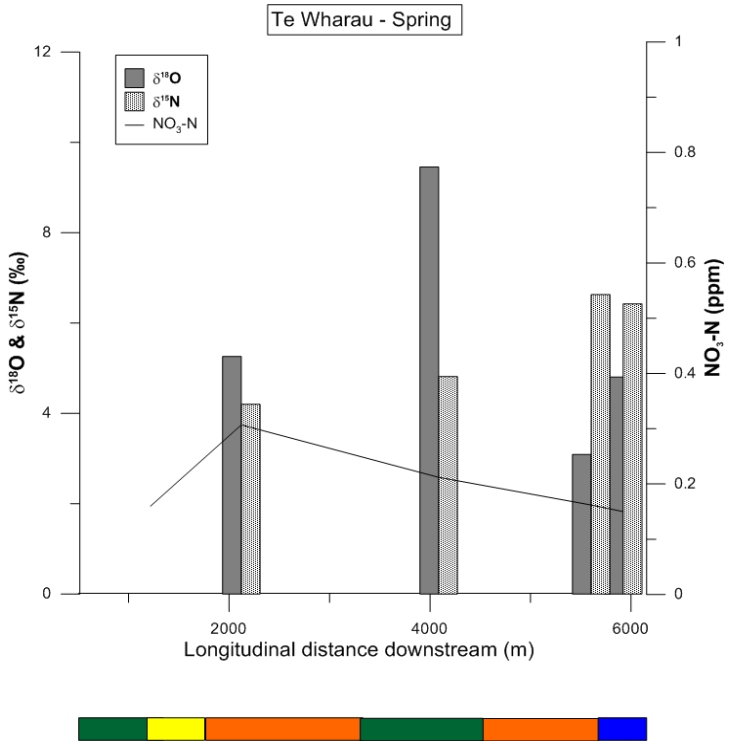
By replicating this study over six catchments, I was able to investigate longitudinal changes in nitrate $\delta^{18}O$ & $\delta^{15}N$ values and nitrate-N concentrations down the length of the catchment. Overall there was a significant relationship between nitrate – $\delta^{15}N$ values and the sampling site's longitudinal position within the catchment (P = 0.010); as water moved down a catchment, nitrate-N became more enriched in ^{15}N. There was no longitudinal relationship with nitrate – $\delta^{15}N$ and $\delta^{18}O$ values however (P = 0.257). Longitudinal patternns in nitrate concentrations and isotopic values down catchments were subjectively investigated for within four of the study catchments (fig. app. 2). Sufficient isotopic data (i.e. more than 4 sites down the length of a catchment) was only available to collected from 7 catchment sampling expeditions. These were: the Te Wharau catchment during summer, autumn and spring sampling (figure 6 a, b and b respectively); the Pawsons catchment during autumn and spring (figure 6 d and e respectively; Hukaika and Narbey catchments both only having autumn sampling available (figure 6 f and g respectively). Because of the reduced dataset, I lacked statistical power to analyse longitudinal versus land-use transition effects. Having no longitudinal patternns in nitrate – $\delta^{18}O$ values or nitrate concentration meant longitudinal analysis was able to detect land-use changes as the mater moved downstream.

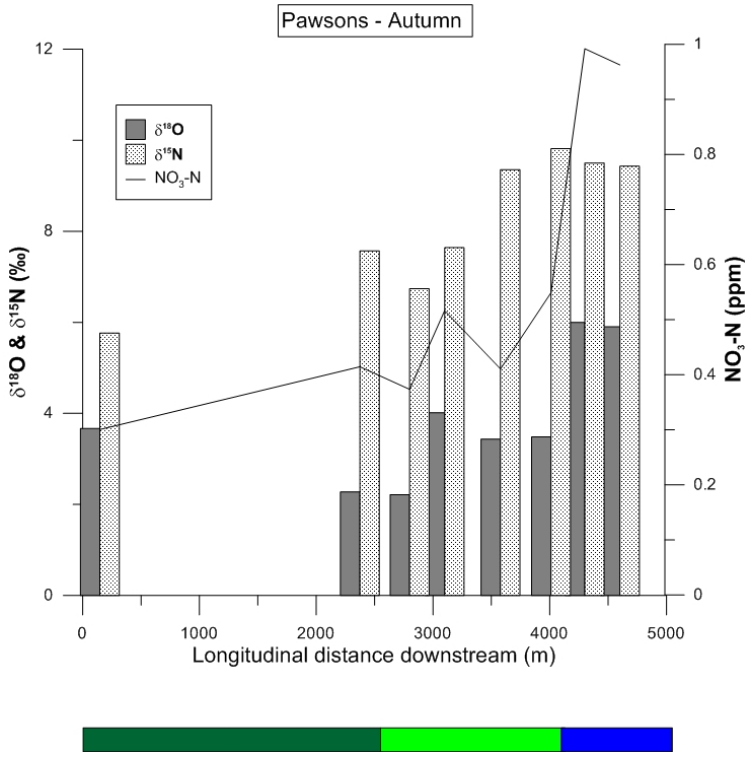
The two starkest land-use transitions occurred between dairy and golf course reaches within the Pawsons catchment during spring (fig. 6 e) and when moving from dry-stock to native regeneration reaches within Te Wharau catchment during autumn sampling (fig. 6 b). The dairy – golf course transition is marked by an increase in the nitrate concentration and a decrease in both the $\delta^{15}N$ and $\delta^{18}O$ nitrate isotope values. This patternn is contradictory to the overall longitudinal enrichment effect on

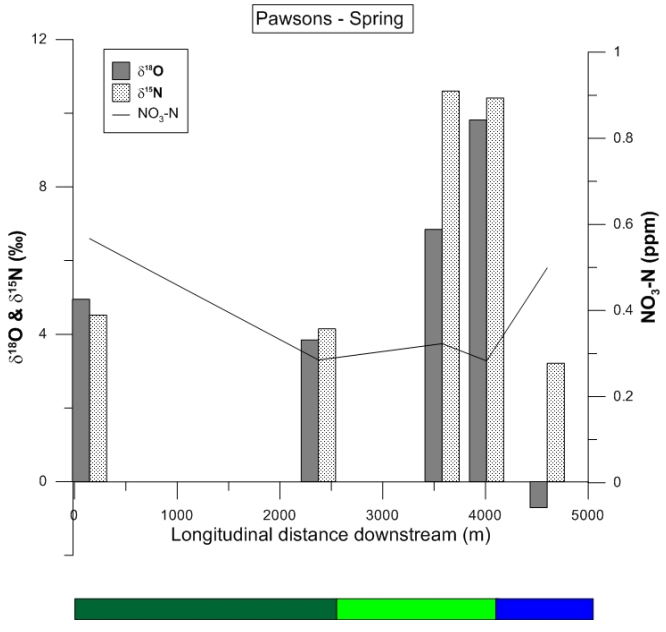
nitrate – $\delta^{15}N$ values, suggesting that this change is nitrate concentration and isotopic values is caused by land-use transition (i.e. nitrate source changes). With little change in the nitrate concentration, the nitrate $\delta^{15}N$ and $\delta^{18}O$ decrease markedly when in the native regeneration site before increasing again on return to a dry-stock reach again downstream during autumn in the Te Wharau catchment (fig. 6 b). In the two aforementioned instances, the observed nitrate isotopic changes with land-use were not consistent over sampling intervals (i.e. season) (e.g. comparing fig. 6 a & c to 6 b, and 6 d to 6e). This indicates likely seasonal differences in the importance of different land-uses on catchments' N-cycles. The one catchment sample which was conducted over a single continuous land-use (fig. 6 f) displayed low nitrate – $\delta^{15}N$ values (~ -0.5‰) within the site closest to the stream origin after which values stayed constant around 4‰. Nitrate – $\delta^{18}O$ values stayed constant around 4 – 5‰ (fig. 6 e) throughout the length of the catchment.

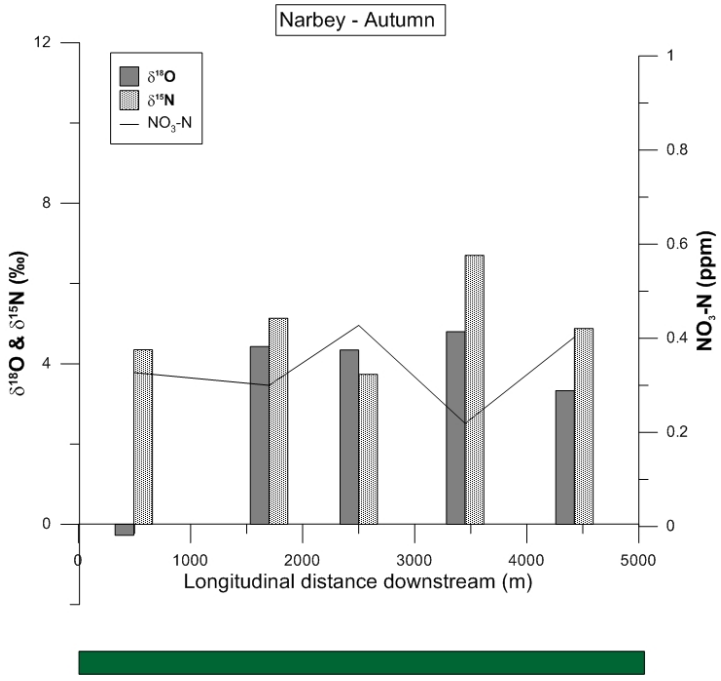



Te Wharau - Autumn
δ18O
δ15N
NO3-N
δ18O & δ15N (‰)
NO3-N (ppm)
Longitudinal distance downstream (m)


Te Wharau - Spring
δ18O
δ15N
NO3-N
δ18O & δ15N (‰)
NO3-N (ppm)
Longitudinal distance downstream (m)


Pawsons - Autumn
δ18O
δ15N
NO3-N
δ18O & δ15N (‰)
NO3-N (ppm)
Longitudinal distance downstream (m)
0
1000
2000
3000
4000
5000
0
4
8
12
0
0.2
0.4
0.6
0.8
1


Pawsons - Spring
δ18O
δ15N
NO3-N
δ18O & δ15N (‰)
NO3-N (ppm)
Longitudinal distance downstream (m)
0
1000
2000
3000
4000
5000
0
4
8
12
0
0.2
0.4
0.6
0.8
1


Narbey - Autumn
δ18O
δ15N
NO3-N
δ18O & δ15N (‰)
NO3-N (ppm)
Longitudinal distance downstream (m)
0
1000
2000
3000
4000
5000
0
4
8
12
0
0.2
0.4
0.6
0.8
1

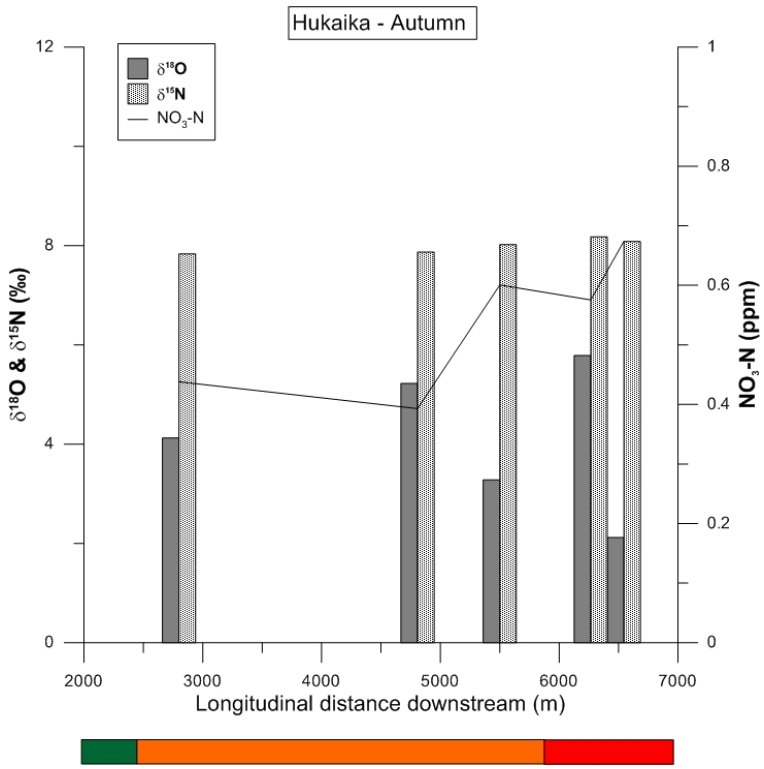

Hukaika - Autumn
δ18O
δ15N
NO3-N
δ18O & δ15N (‰)
NO3-N (ppm)
Longitudinal distance downstream (m)
2000
3000
4000
5000
6000
7000
0
4
8
12
0
0.2
0.4
0.6
0.8
1

Figure app. 2: longitudinal sampling of nitrate – $\delta^{15}N$ and $\delta^{18}O$ values and nitrate concentration (NO_3-N ppm) down catchment length. A) Te Wharau – summer, b) Te Wharau – autumn, c) Te Wharau – spring, d) Pawsons – autumn, e) Pawsons – spring, f) Narbey – autumn, g) Hukiaka – autumn). Colour bar indicates the stream length coverage of different land-uses using the common colour-coding system (dark-green: native regeneration, orange: dry-stock, lime-green: dairy, yellow: gorse and broom, horticulture: red and royal blue: golf course). Distance from cartographic source indicates the distance downstream of the sampling site from the stream origin as defined by stream length in the *Ministry for the Environment* land cover database)

Discussion of Longitudinal patternns

Overall nitrate concentrations and $\delta^{18}O$ isotopic values showed no downstream longitudinal relationship. Longitudinal position within a catchment has previously been shown to be a poor predictor of nitrate concentration (Young et al. 2005). Patternns of longitudinal nitrate accumulation generally only recorded in systems which are saturated (Carpenter et al. 1998). Nitrate – $\delta^{15}N$ values on the other hand, did display a positive longitudinal relationship. Longitudinal increases in nitrate – $\delta^{15}N$ values has been recorded before and attributed to denitrification (Diebel and Vander Zaden 2009). As discussed earlier, this longitudinal increase in nitrate – $\delta^{15}N$ and $\delta^{18}O$ values is most likely to primarily driven by biological uptake as opposed to denitrification in this study system. The approximately 2 km reach of continuous dairy farming down the length of catchment (high nitrate concentration) which was longitudinally sampled provides an interesting comparison to the longitudinal sampling down the length of the continuously forested catchment (low nitrate concentration). Both catchments were sampled during autumn. In the forested catchment the nitrate – $\delta^{15}N$ immediately increases before remaining relatively static down the length of the catchment while the concentration and $\delta^{18}O$ value changed erratically. On the other hand, nitrate within the dairy reach became continuously enriched in both $\delta^{15}N$ and $\delta^{18}O$ and the concentration also increased. The longitudinal dual isotopic enrichment observed

within the dairy reach area similar to those observed within areas receiving excess nitrate (Ohte et al 2008; Itoh et al. 2011).

Several distinct stable isotopic and concentration changes were observed between land-use transitions. This the strong effect of land-use on stream nitrate cycling and supply. Amongst the most distinct examples are the transitions from dry-stock through native regeneration then to golf course along the golf course reaches in the Te Wharau catchment during autumn and the transition from dairy farming to golf course reaches in the Pawsons spring sampling. A drastic decrease in $\delta^{18}O$ during the reach of native regeneration without and significant decrease in the $\delta^{15}N$ was noted in the Te Wharau catchment during autumn sampling. Higher nitrate concentrations were observed in the gorse and dry-stock reaches upstream. Low $\delta^{18}O$ nitrate values are typically attributed to microbial nitrification (Kendal 2007). That there is little change in the $\delta^{15}N$ nitrate signature from upstream isotopic values suggests that there is little isotopic discrimination (fractionation) during uptake; indicative of system with relatively tight nitrogen cycling. The low $\delta^{18}O$ and moderate $\delta^{15}N$ signature is indicative of nitrification (Kendal 2007). From interviewing the golf course greens keeper, it was discovered that the greens were amended with an ammonium based fertiliser in the spring in order to 'boost' grass growth on the greens. While an isotopic signature is not available for the fertiliser used on this golf course, the nitrate isotopic signature falls in the range microbial nitrification. While the $\delta^{15}N$ nitrate isotope signature is towards the high end of the expected range of values expected for nitrified fertiliser (Kendal 2007), ammonium volatilisation commonly enriches ammonium bodies in ^{15}N (Robinson 2001). The longitudinal observations from these two catchments suggests that land-use can alter the scale of nutrient cycling (i.e. an exporting reach vs. one with tight nutrient cycling) at a reach (<1km) scale. It is likely that the rapid responses to land-use change observed here are related to the N-limited nature of the study area.

Other studies looking at catchments which are assumed to be P-limited appear to exhibit a far higher degree of longitudinal nitrate accumulation and isotopic source mixing (Anisfeld et al. 2007; Ohte et al 2008; Itoh et al. 2011). The dramatic reduction in nitrate – $\delta^{18}O$ seen in the Te Wharau catchment as the moved from the dry stock reach into the native regenerating forested one was most likely due to increased in-stream nitrification (Kool et al. 2011).

Appendix 3: Longitudinal $\delta^{13}C$ enrichment observed in invertebrates.

Invertebrate $\delta^{13}C$ values showed a significant positive increase downstream. Changes in consumers' isotopic signatures can arise primarily from one of two reasons; firstly switching to another food source, secondly the isotopic signature of the food source itself has changed. Traditionally the $\delta^{13}C$ signature of a consumer has been seen as a static indicator of the trophic channel supporting that organism/population. This is because fractionation rates during photosynthesis are the primary control on autotrophs' $\delta^{13}C$ signature (Finlay and Kendall 2007). This is assuming that CO_2 used in photosynthesis is in equilibrium with the atmosphere (Finlay and Kendall 2007). Grey et al (2010) showed that stream autochthonous production within spring upwelling areas can be ^{13}C deplete due to dissolved inorganic carbon (DIC) inputs from ground water. Through the significant longitudinal $\delta^{13}C$ enrichment ($P = 0.0028$), my data indicates that headwater sites are sequestering potentially significant amounts of CO_2. Additionally, within several of the headwater sampling sites, mixing model analysis showed that the isotopic signatures of some of the consumers plotted outside of the range of the three primary resources. In all cases the unaccounted consumers had isotopic signatures more deplete than that of the measured resources; indicating that they were indeed consuming groundwater derived subsidies. Butman and Raymond (2011) demonstrate that lotic systems can be significant sources of CO_2 emissions. However, my results indicate that substantial portions of this CO_2 may in fact be sequestered in the stream and locked up in organic matter. Investigating how this in-stream sequestration and interactions with nitrogen cycling impacts riverine CO_2 emissions warrants future study.

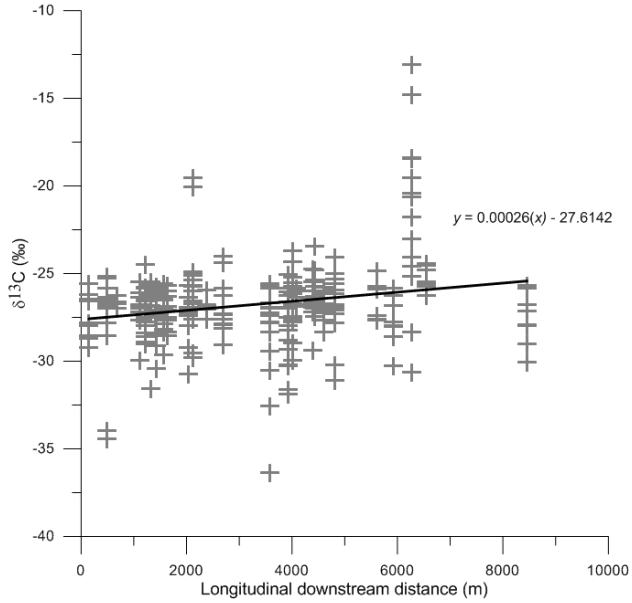


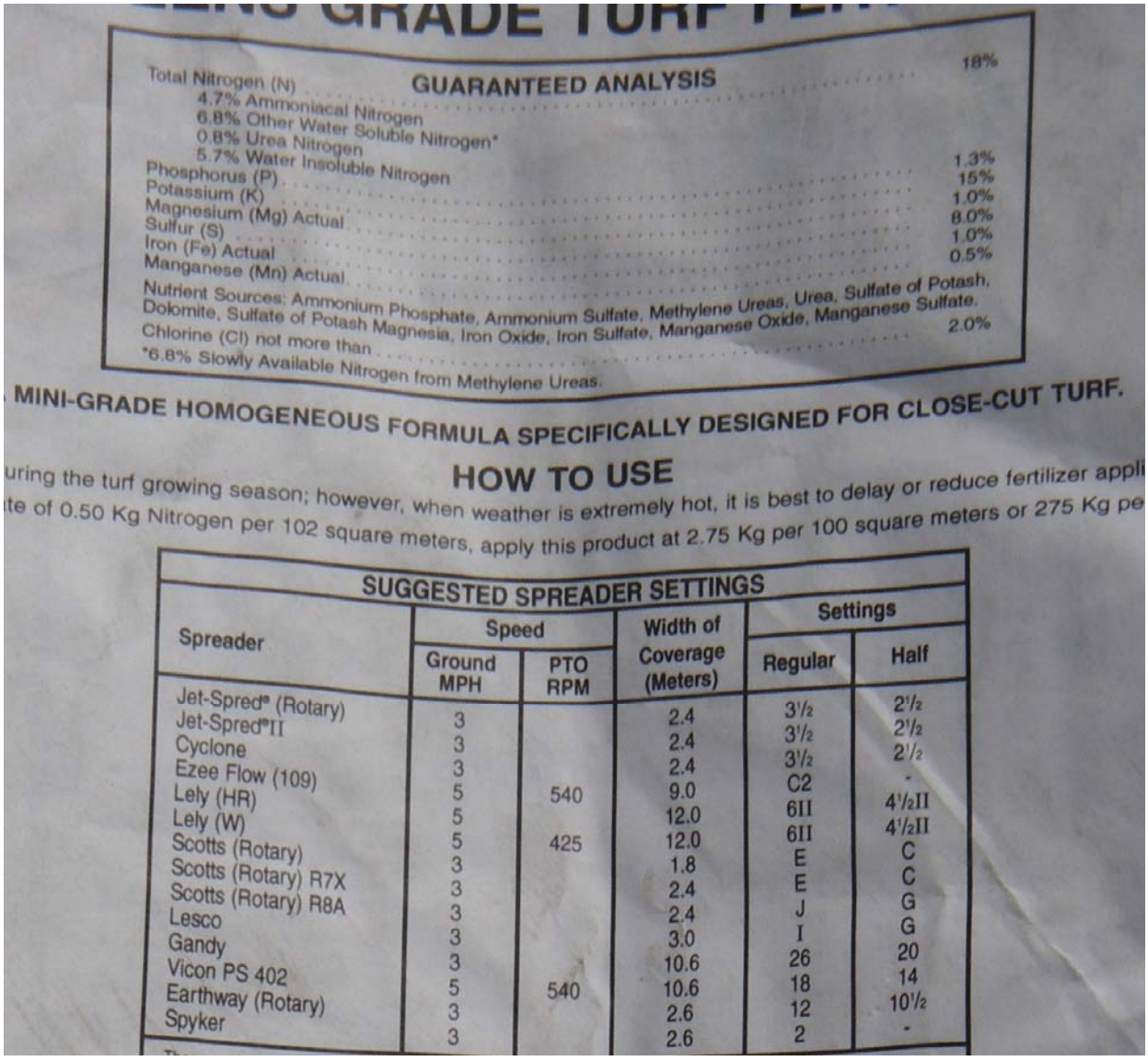
Figure App. 3: invertebrate $\delta^{13}C$ enrichment over the longitudinal distance downstream. P = 0.0028, n = 291

Appendix 4: Photographs of typical conditions associated with the studies land-uses on the Banks Peninsula

Looking up the Waiake catchment; a typical Banks Peninsula setting showing the step catchment and volcanic topography. The grass cover within the picture is an example of typical dry stock agricultural land in the area. The vegetation cover in the picture centre is regenerating native forest. Again this is typical of the area both in its maturity (primarily mature Kanaka) and its location; within-stream valleys in a low intensity agricultural setting

Dairy pastures in Pawsons Valley. The photo was taken within two weeks of the previous picture of dry stock pastures (February 2010). Note the difference in grass productivity

A first order regenerating native forest stream within the Narbey catchment. The dense canopy cover with mixed-species sub-canopy and fern ground cover is typical native forested stream on the Banks Peninsula and throughout New Zealand



GRADE TURF

GUARANTEED ANALYSIS

Total Nitrogen (N)	18%
4.7% Ammoniacal Nitrogen	
6.8% Other Water Soluble Nitrogen*	
0.8% Urea Nitrogen	
5.7% Water Insoluble Nitrogen	1.3%
Phosphorus (P)	15%
Potassium (K)	1.0%
Magnesium (Mg) Actual	8.0%
Sulfur (S)	1.0%
Iron (Fe) Actual	0.5%
Manganese (Mn) Actual	

Nutrient Sources: Ammonium Phosphate, Ammonium Sulfate, Methylene Ureas, Urea, Sulfate of Potash, Dolomite, Sulfate of Potash Magnesia, Iron Oxide, Iron Sulfate, Manganese Oxide, Manganese Sulfate.

Chlorine (Cl) not more than 2.0%

*6.8% Slowly Available Nitrogen from Methylene Ureas.

MINI-GRADE HOMOGENEOUS FORMULA SPECIFICALLY DESIGNED FOR CLOSE-CUT TURF.

HOW TO USE

uring the turf growing season; however, when weather is extremely hot, it is best to delay or reduce fertilizer appli
te of 0.50 Kg Nitrogen per 102 square meters, apply this product at 2.75 Kg per 100 square meters or 275 Kg pe

SUGGESTED SPREADER SETTINGS

Spreader	Speed		Width of Coverage (Meters)	Settings	
	Ground MPH	PTO RPM		Regular	Half
Jet-Spred® (Rotary)	3		2.4	3½	2½
Jet-Spred®II	3		2.4	3½	2½
Cyclone	3		2.4	3½	2½
Ezee Flow (109)	5	540	9.0	C2	-
Lely (HR)	5		12.0	6II	4½II
Lely (W)	5	425	12.0	6II	4½II
Scotts (Rotary)	3		1.8	E	C
Scotts (Rotary) R7X	3		2.4	E	C
Scotts (Rotary) R8A	3		2.4	J	G
Lesco	3		3.0	I	G
Gandy	3		10.6	26	20
Vicon PS 402	5	540	10.6	18	14
Earthway (Rotary)	3		2.6	12	10½
Spyker	3		2.6	2	-

Common fertilizer applied to the two golf courses investigated in the study

Looking across to the western side of the head of the Te Wharau catchment from Lyttelton Harbour; the dull yellow is gorse and broom in bloom